AQUAFISH CRSP

TECHNICAL REPORTS
INVESTIGATIONS 2009–2011
VOLUME 1

AUGUST 2012

Aquaculture & Fisheries
Collaborative Research Support Program
Management Entity
Oregon State University
418 Snell Hall ♦ Corvallis, OR 97331-1643 ♦ USA
aquafish@oregonstate.edu

USAID FROM THE AMERICAN PEOPLE

Oregon State University OSU

AQUAFISH COLLABORATIVE RESEARCH SUPPORT PROGRAM

AQUAFISH CRSP TECHNICAL REPORTS: INVESTIGATIONS 2009–2011, VOLUME 1

Program activities are funded by Cooperative Agreement No. EPP-A-00-06-00012-00 from the United States Agency for International Development (USAID), and by participating US and Host Country institutions.

Disclaimers

The contents of this document do not necessarily represent an official position or policy of the United States Agency for International Development (USAID). Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use on the part of USAID or the AquaFish Collaborative Research Support Program (CRSP). The accuracy, reliability, and originality of work presented in this report are the responsibility of the individual authors.

Cover Photo

Buyers haul off a basket of freshly caught fish from Tri An Reservoir in Vietnam, where AquaFish CRSP researchers are assessing the impacts of fish stocking on wild fish populations. Photo by Peg Herring, Extension & Experiment Station Communications, Oregon State University.

This publication may be cited as:

AquaFish Collaborative Research Support Program. August 2012. Technical Reports 2009–2011, Volume 1. AquaFish CRSP, Oregon State University, Corvallis, Oregon, 573 pp.

AquaFish CRSP Management Entity
Oregon State University
418 Snell Hall ♦ Corvallis, OR 97331-1643 ♦ USA

Table of Contents: Volume 1

Evaluation and Improvement of Production Technology in Uganda: Case Studies of Small-Holder Cage Culture in Watershed Reservoirs and as an Alternative Livelihood for Fishers

Production System Design and Best Management Alternatives/Study/09BMA01AU

Emily Stutzman Jones and Joseph J. Molnar
Auburn University
Auburn, Alabama, USA

Gertrude Atukunda and John Walakira
National Fisheries Resources Research Institute
Kajjansi, Uganda

ABSTRACT

Aquaculture development commentary supports the formation of fish farmer associations or producer organizations as avenues for cultivating small- and medium-scale commercial farmers. However, little is known about the types of associations that facilitate commercialization. This research presents four qualitative case studies, based on semi-structured interviews, profiling existing associations of commercial fish farmers in Uganda. We conclude that the umbrella organizations under which local fish farmer associations vertically align themselves have important implications for fish farmer production. Aquaculture-specific umbrella organizations contribute to the success of local member associations more than general umbrella organizations do. Successful fish farmer associations accept government assistance only when it directly improves their fish farm operations. Other farmer groups seemed to wait for direct subsidization. Training fish farmers, providing quality information, cost sharing, and advocating for the aquaculture sector, not donor seeking, are the top priorities in productive fish farmer associations. Part I of this report summarizes the four case studies; Part II summarizes the results of the cage culture trials.

INTRODUCTION

Improving the livelihoods, nutrition, and opportunities of the rural poor is a central goal of development efforts, particularly the aquaculture sector. These efforts target smallholder farmers, who make up 70 percent of the African continent's population. Most rural farmers make their livelihoods from small-scale, mixed enterprises, producing first for home consumption and second for sale (Brummett et al. 2008:375). The prevailing approach to aquaculture development in Sub-Saharan aquaculture between the 1970s through the 1990s targeted the rural poor mainly by supporting tilapia and the African catfish as culture species. The FAO, the Peace Corps, and USAID largely centered their efforts on small-scale, limited input, integrated fish farming for improved household fish consumption and income with often disappointing or inconsistent results (Brummett et al. 2008:375, Moehl 2006:v). Currently, 90 percent of African fish farmers fall into this small-scale or artisanal category (Brummett et al. 2008:380).

Gains from small-scale, integrated fish farming systems generally are not captured in official statistics. Nevertheless, rural food security advances through increasing small farm production levels (Brummett et al. 2008:375). However, small-scale, integrated fish farming operations realize little cash gain due to the small quantities and low production intensity, that is, the weight of fish produce per unit area (Brummett et al. 2008:375). Increasing production intensity is a central goal in aquacultural development. Several factors work against the continued promotion of subsistence-level fish farms, including the expense of training and extension and the low expectations for economic returns from this diversified farming system (Brummett 2008:383).

Technical aquaculture experts have long understood that success in aquaculture hinges on human factors (Moehl 2006). Sociologists involved in aquaculture development find that personal commitment to fish farming is perhaps a more vital predictor of success than technical knowledge (Molnar et al. 1985). We have learned how commitment supports sustained attention to technical matters for individual farmers, but increasingly groups are used as mechanisms for extending technical knowledge, engendering mutual support, and sharing burdens such as surveillance to prevent theft and harvest of ponds. The purpose of this paper is to describe organizational and sociological factors that influence the success of commercial aquaculture in Uganda by examining four existing fish farmer associations. Each association relies on different coping strategies and mechanisms of affiliation to realize its fish farming objectives, albeit with different degrees of success.

PART I: CASE STUDIES OF SUBSISTENCE AQUACULTURE

Subsistence aquaculture is being re-evaluated and the commercialization of agriculture as a whole is the present focus of the Food and Agriculture Organization of the United Nations (FAO) in Sub-Saharan Africa and the Ugandan government's national policy as well. Several donor organizations and the FAO, are working to transform selected farmers from small-scale to commercial fish farm operators. The premise is that fish farmers who operate mainly for profit and can be the driving force behind aquaculture infrastructure development, including the production of quality fish fingerlings or "seed" and the use formulated feed in production (as opposed to reliance on pond fertilization and generally inadequate farm-produced feeds). The abiding characteristics of these profit-oriented farmers are yet to be realized, as there are currently only 200 such Ugandan fish farmers. A focus on commercial operators coincides coinciding with the Ugandan government's promotion of fish exports (Mwanja 2005). [1]

Fish farmer associations are a key factor in establishing a viable commercial aquaculture sector in Sub-Saharan Africa (de Selingy 2006, Moehl 2006, Hecht 2005). A farmer association is defined as a conglomeration of individual farmers and/or fish farming groups joined for the purpose of more effective coordination of activities, and for established capacities to address several constraints and limitations faced by members. They are primarily social organizations and members of an association do not own joint fish ponds under the umbrella of the association (Moehl 2006). Some beneficial roles which fish farmer associations can play include influencing policy and regulations, providing technical services, facilitating market access, aiding in aquaculture research programs, providing extension services, developing and encouraging adherence to codes of conduct or better management practices, extending credit to member farmers, and facilitating knowledge-sharing (Hecht 2005, de Selingy 2006, Mosher 1966). In Africa, such entities are often the beginning points for developing a national industry.

Despite the long lists of roles for fish farmer associations to perform, no framework or set of guidelines exists for how effective associations can be created (Moehl 2006). In fact, many fish farmer associations are described as ineffective or short-lived, and links between donor funding and association creation are common, as promises of gifts often accompany injunctions to form farmer associations; in these cases,

[1] Aquaculture now is seen as a private-sector led enterprise that is technically sound, economically profitable, socially acceptable, and environmentally sustainable with the state playing a role as a facilitator and monitor (Brummett et al. 2008, de Seligny 2006). Commercialization of aquaculture need not exclude small holders; the distinction is more a reflection of motivation, goals, and business and management practices than scale (Brummett et al. 2008:375, Moehl 2006). In comparison to artisanal, integrated fish farmers, the small-to medium-scale commercial farmers typically build more ponds, use more technology, employ laborers, purchase fingerlings, use commercial feeds, and employ nonlocal business strategies. Commercial operators transport fish to urban markets where customers pay cash for fish (Brummet et al. 2008:380). Producers and consumers benefit from the commercialization of aquaculture.

associations commonly disintegrate after incentives disappear (Hecht 2005, Moehl 2006, Harrison 1996). There are few surviving instances of thriving fish farmer associations to cite as examples (Moehl 2006).

Nonetheless, government and donor interest in fish farmer associations remains strong because of the need to reach large numbers of adopters, using farmer field schools and other extension models to leverage the efforts of tr**a**iners and extension personnel (Moehl 2006). Larger numbers of beneficiaries participate in investments in pond construction, feed subsidies, and seed stock supply. A growing focus on commercialization necessitates that farmers have all available tool for success, as the financial stakes are higher than with previous subsistence efforts. Associations can provide some of the tools, in the form of knowledge, access to quality inputs, and relationships with aquaculture technicians, which individuals need to succeed as commercial fish farmers. Emerging commercial fish farmers, who have the desire to learn new techniques and improve production, are a target group for successful fish farmer association development (Hecht 2005). The case studies elucidate the way these efforts actually are realized in rural African communities.

PART II: CAGE CULTURE TRIALS

Cage culture is a new aquaculture technology in Uganda that involves the utilization of lakes, rivers and large water reservoirs. Most of these natural resources are over exploited due to the massive fishing pressure aimed at maximizing catches. As a result, many of the aquatic resources are being depleted. Therefore, cage culture can play the role of providing an alternative form of livelihood for fisher communities in order to practice aquaculture alongside sustainable fishing. Its advantages include ease with handling fish, high stocking densities, ease of controlling predators, utilizes less labor and higher turnover of profits. Some of the barriers of successful cage culture include: high investment costs combined with difficulties in access to credit and/ necessary materials, unavailability of cost effective high quality fish seed, theft of fish, problems concerning use of areas considered as public domain and challenges in marketing of cage reared products (FAO, 2004).None the less, cage culture is a venture that is attracting investment interest by a cross section of actors right from community based fisher groups to foreign commercial investors in Uganda. This is largely because it has the potential to produce large quantities of fish for domestic markets and for export.

USAID supported research as well as the fisheries section of National Agricultural Research organization conducted initial cage culture trials and demonstrations. Results of the research showed the possibility of small holder groups to engage in cage culture. In particular, fishers showed keen interest in engaging in cage culture mainly because many of them were losing employment due to reduced wild fish caged that were no longer viable. Towards the end of the FISH project, at least two groups of fishers had started the required formal process to enable them obtain permits for engaging in cage culture on L. Victoria. By the year 2007, the groups had obtained the permits. Permits for cage culture have to be obtained from the Ministry of Agriculture Animal industry and Fisheries (MAAIF), the National Environment Management Authority (NEMA) and the Directorate of Water Resource management of the Ministry of Water Lands and Environment.

Although the project initially planned to work with four farmer groups in four different localities, only one group (Jinja United) was finally eliminated due to financial limitations. In addition, one group got into another arrangement with government while the other two faced problems of group cohesion and could not continue with the activities.

Following acquisition of the permits, the group members were ready to start but did not have enough money to cover the key cost i.e. cages. One group obtained financial support from government so AquaFish CRSP investigators opted to work with the other group that had raised some capital from their own savings. Discussions were held with group members and a Memorandum of Understanding was

drawn and signed by the two parties. It was agreed that the project would provide the cages and technical advice while the group members would provide the some of the fingerlings at stocking and all the labor required in feeding, sampling and ensuring security. The Memorandum of Understanding (MoU) also spelled out details of responsibilities of the two parties and their expectations. With technical assistance by AquaFish CRSP investigators, a financial management plan and draft enterprise budget were developed with the group.

During the investigation and demonstration, emphasis was put on cash flow management. This was in order to demonstrate that the group can source capital and with good management be able to make profits from cage culture.

As result of the increasing fishing pressure, aquatic resources are at a risk of depletion. Therefore, evidence that cage culture is a profitable venture is a key aspect in providing information that can transform fisher communities to practicing aquaculture as an alternative form of livelihood. The study aimed at providing evidence of cage culture as a profitable venture and information on some of the management aspects that should be emphasized.

The number of fishers on Lake Victoria has increased tremendously since 2000 and the increased pressure on the fishery has led to adoption of illegal and highly destructive fishing methods. Moving traditional fishers to farming has often been cited as near impossible. However, many of Uganda's fishers are new comers to fishing because they were not able to subsist on agriculture. This group of people could more easily be moved into fish farming compared to groups who have been fishing for several generations.

METHOD

PART I: CASE STUDIES OF SUBSISTENCE AQUACULTURE

Case studies of four fish farmer organizations in diverse areas of Uganda were conducted during January and February 2010. Yin defines a case study as an "… empirical inquiry that investigates a contemporary phenomenon within its real-life context, especially when the boundaries between phenomenon and context are not clearly evident" (2008:13). Multiple case study analysis is a research method that looks carefully at persons and operations at several locations in order to understand a complex situation (Stake 2006). Evidence from multiple case studies is likely to be stronger than that of single case studies (Yin 2008:19).
' previous professional connections the associations had made with the Aquaculture Research and Development Centre, Kajjansi (KARDC), a branch of The National Fisheries Resources Research Institute (NaFIRRI). Recruiting focus group research participants from associations where potential participants seek services is one method for recruiting research participants (Hennink 2007:102). All three associations have donor project relationships. We intended to conduct focus group interviews with a sample of members from each aquaculture group. However, in the cases of "The Unaccountable Leaders" and "The Helping Hands," this was not possible, as the fish farmer association leaders were not cooperative in arranging focus group meetings. In these situations, data emanate from semi-structured interviews with the fish farmer association's leaders, extension officers, and other informants.

We identified "The Cooperative Society," an organization without direct development project ties or previous contact with the collaborating fishery officers. Contact with this organization came through a fish farmer organizer met at Uganda's Annual Fish Farmer Symposium and Trade Show. The case provides a contrasting comparison, as the other groups are representative of the type of fish farmer associations that maintain contact with government researchers, and "The Cooperative Society" does not. Events, meetings, and conferences are also useful venues for recruiting focus group research participants (Hennink 2007:101). The contact is the organizer and chairman of the Uganda Fish Farmers Cooperative Alliance. "The Cooperative Society" is one of the groups organized under the Uganda Fish Farmers

Cooperative Alliance umbrella. We examine each case in the context of the guiding issues of internal dynamics and relative success in the targeted technical activities.

PART II: CAGE CULTURE TRIALS

Initial work was to verify the appropriateness of the site allocated to the farmer group. GPS readings were taken and water quality parameters of Oxygen and temperature were also recorded. The site is close to Kirinya prisons, Jinja at the shore of Lake Victoria.

Table 1. Parameters of cage sites

Distance from shore line	Elevation	GPS readings		Temperature	Oxygen	Cloud cover
193M	1143	N0.41346	E033.23247	26 ^{0}C	3.5 Mg/l	80%

Cage installation. Two cages (2M by 2M by 2M) were installed on the selected site. Each cage was stocked with 2030 Sex reversed *Oreochromis niloticus* of average 4g that were obtained From Source of the Nile Fish farm. The initial plan to have the fry nursed in ponds was not performed because the farmers' ponds were not in good condition.

Management. Training in the management of cages was conducted a day before stocking the cages. The training was attended by Jinja United farmer group members (4 women and 6 men). The training was delivered by the AquaFish CRSP project team supplemented with technical assistance by a technician at SoN fish farm. The training covered the following topics:

- Feeding techniques
- Collecting and recording mortalities
- Record keeping (technical and financial)
- Group dynamics

The second training was conducted at the time of sampling fish at month three (March 2011. Besides Jinja United farmer group members, this training included some members of the Masese NAADS farmers group and some members from the surrounding community. Besides discussing sampling results, the issue of the need to use a stronger net cage was discussed since it had been realized that fish had escaped from one of the cages. Feed amounts were administered based on fish size and adjusted depending on fish response.

Data collection. Group discussion interviews were initially held with the farmer group member to obtain information about the history and organization of the group. This exercise was part of the field work carried out by Masters student from Auburn University. The next round of group discussions involved farmer group members (4 women and 6 men) and some members from the surrounding community.

Sampling of fish was carried out to monitor fish growth and to determine the right amount of feed to be administered during the subsequent month. Members of the farmer group kept records of cost of feed fed to the fish, feeding response and any fish mortalities encountered. Other records the group kept included members' cash contributions to the expenses and a roster of members' personal visits and activities carried out at the site.

RESULTS

Two associations are beginning to operate cage culture aquaculture systems, one is a fingerling producer, and the members of a fourth farm fish in ponds. In order to facilitate comparisons and analyses of factors that make fish farmer associations successful at improving their member farmers' fish production, the cases have been ordered from fish farmer associations with the lowest fish production to the entity whose members produce the most fish.

CASE STUDY ONE: "THE UNACCOUNTABLE LEADERS"

In western Uganda, bordering Queen Elizabeth National Park is a group of individuals who operate cages on the deep inland waters known as Uganda's crater lakes. They operate under a regional environmental conservation umbrella group. The environmental conservation umbrella group has 69 members and nine people in leadership positions, including a chairperson, vice chairperson, treasurer, secretary, project coordinator, and committee members.

The environmental conservation organization became involved in fish farming with cages through the project coordinator in 2008. As part of a five-year countrywide aquaculture development project, a subset of this association received some training, and project staff conducted water quality tests for 13 lakes, which demonstrated eight viable for fish farming based on indicators including dissolved oxygen and hydrogen sulfide levels. One lake was selected as an experiment and five cages were placed on the lake.

Cage culture. Of 70 people who came to learn about fish farming (some of whom maintain their own fish ponds), ten were selected to manage the cages on the selected lake. This operation was designated as a model farm. The group maintained the tilapia fish in the cages through two production cycles. But, due to a lack of feeds, the cages are currently empty.

In the view of the project coordinator, the first harvest was a success, though two of the five cages had problems just before harvest, which rendered them useless. One cage's top had not been latched correctly, so the fish escaped. Another's net was torn, possibly by otters. The other three cages were harvested and given to the people participating in the project in order to demonstrate the success of the venture as well as to establish that farmed fish tastes like wild-caught fish, as many people were skeptical of farmed fish.

The second harvest was also a success, though only two cages were in use. After harvest, the fish were salted and sun-dried, a low-cost preservation and value-addition method, and sold to traders from the Democratic Republic of the Congo. The project coordinator said, "We only had two cages because we had no feeds and the cages were getting old, and the feeds we were using were expired. Feeds are very expensive." The cages have since been repaired.

Resources necessary for production are currently the problem, as members cannot afford the investment. The chairman said, "People are willing to participate, but pooling resources is not affordable for the members, though a few members can."

Leadership. The honesty of the two leaders of the association was called into question during the discussion of the group's first harvest. It remains unclear why the fish from two of the five cages in the second production cycle disappeared. When asked if theft rather than an animal predator or unlatched lid could have led to the empty cages, the project coordinator said, "They don't steal from the cages because there is 24/7 monitoring." Theoretically, a full-time guard would have seen problems with an unlatched lid and an animal. Additionally, it became clear that the project coordinator never asked the members involved in fish culture to come to participate in interviews. A collaborating researcher conjectured that the project coordinator's actions reflect the members' distrust of him as a leader. Also, as the government research station plans to provide financial assistance to the fish farmers of this organization, the project

coordinator sought to prevent his members from meeting the actual source of the funding, perpetuating the allusion that the project coordinator himself is the supply line of assistance. The project coordinator spearheaded the fish farming efforts and is an aspiring politician, though currently not holding office.

There is little evidence of meaningful interaction between the fish farming members of this association and its leaders. The general meeting scheduled to take place once a year did not occur last year or this year. Executive meetings attended by those in leadership positions occur as necessary. Technical meetings, which include the people involved in a specific project such as fish farming, took place once a week during production. During these technical meetings topics such as feed issues, the age and size of the fish, and problems that have arisen are discussed. Transparency with this core group of people involved in the fish farming is a challenge, especially as other members see the profits and become jealous. The inequality of benefit distribution is a source of members' jealousy. The project coordinator, who facilitated the donations of feed and equipment as well as invested some of his own money, explains the distribution of benefits. He says, "People who have put in big investments must have the lion's share."

It also seems that the leaders are intentionally unaccountable to the members. When asked if members pay dues, the chairman said, "They are doing voluntary work hoping to get a share of the proceeds. We have people who are ready to pay money to be members but we are not signing them up because we cannot take their money when there are no feeds because they will be asking 'What is happening with our money?' We have a very big number [who are interested] but we cannot accommodate [more members]." Thus, the members take no financial risk to purchase the necessary feeds and reap no reward. The project coordinator has a vested interest in limiting the risk that his members take: To have a failed harvest into which members invested their own resources would harm the project coordinator's reputation and potentially decrease his political support in future elections.

CASE STUDY TWO: "THE HELPING HANDS"

The umbrella regional poverty alleviation organization has a fish farmer association of 88 members. The group's formation was stimulated by the chairman's enthusiasm for fish farming. Additionally, the chairman expressed that he organized the group to meet members' needs and to access funding for projects. Some members own and maintain fish ponds, and others assist with a group pond. Several other charitable organizations have fish pond projects under the umbrella of the regional poverty alleviation organization. The fish farming members of "The Helping Hands" organization are preparing for a transition of emphasis from individually- and group-managed fish ponds to group management of a fish cage culture operation on Lake Victoria. The focus of our study was the structure of effort towards the potential transition to cage culture. Most of the interviewees were leaders of "The Helping Hands."

The fish farmer group typically holds meetings four times a year but gathers more frequently when preparing for a workshop or another unusual event. Currently, the fish farmer subset of "The Helping Hands" is not managing fish production collectively, but the chairman says they are ready to begin as soon as funds are available for that purpose. The chairman says, "As a management structure we have people in place but they are not functional (currently functioning). So the people are ready for when we have the money." The chairman appoints leaders and their responsibilities are based on the individual leaders' expertise. "Whoever has the ability of doing something does it voluntarily for the benefit of the group," states the chairman. This commitment to community service is shared among the group, though to some degree each executive member stands to benefit financially or politically through their involvement in the group's poverty alleviation projects.

Political connections. Under the umbrella of "The Helping Hands," and hence under its chairman, is a regional fish farmers association that encompasses local associations from four districts in eastern Uganda. The chairman unified them, saying, "These groups weren't capacitated (empowered) because

they were singular (working in isolation)." This integration followed a large fish farmer meeting with over 300 attendees organized by the chairman. At the meeting, the President's assistant announced that the chairman would be the one to distribute information and assistance to the fish farmers in this region. Two aspects of this fish farmer meeting reflect the chairman's political pull: the presence of the president of Uganda's assistant and his pronouncement that the chairman of "The Helping Hands" will channel assistance to area fish farmers. Other examples further illustrate the chairman's political power.

The goal of "The Helping Hands" cage culture operation on Lake Victoria is to be a demonstration or model farm, which is a political status, and an achievement for which the chairman will potentially be credited and financially rewarded. In addition, the local government provided the group funds to acquire the necessary permits for operating cages on the lake. The minister of fisheries wrote on "The Helping Hands" behalf to the executive director of NAADS. Each achievement reflects the chairman's access to influential politicians, the essence of political power.

There are at least two perspectives on the political affiliation of the chairman and his fish farming aspirations. In a short-term view, political connections can lead to resources otherwise very difficult to procure, including permits, funding, and support for aquaculture activities. On the other hand, considering goals of sustainability, politicians' goals are often incongruous with the goals of the development of commercial fish farmers.

Cages first. The management approach that "The Helping Hands" organization uses for fish farmer development is rooted in its origins as a collectivity. The chairman says, "After all, it is up to everyone to look after the structure. Management is organized by the group and owned by the group." The group manages community fish ponds and hopes to operate cages with the expectation that profits from these operations will be used to purchase additional cages and inputs for individuals to own their own cages. The chairman says, "At the beginning we feel like we should work as a team. As we grow and begin realizing profits we should support individuals in owning cages. They will be then capable of owning and managing their own cages."

The goal of "The Helping Hands" umbrella group is poverty alleviation and economic development. It appears that the activities and goals of the group are more charity-based than business-oriented. When the chairman was asked why he and his members wanted to be fish farmers, he said, "It is the farming that can help people of different abilities. Fish farming gives a chance to vulnerable groups including women who can't go fishing by boat on the lake but can fish farm. It is an opportunity for the disabled, orphans, and the elderly. Also, fish farming can be done in teamwork. After all, it is up to everyone to look after the structure."

When asked what would evidence the success of his cage culture operations on Lake Victoria, the chairman said, "Being that cage culture is new, we expect that people will realize that it is good. We want to show a demonstration project. In the process of time, people, after learning from us, will apply knowledge on an individual level. They will arrange for their own permits. Success will be proved by individuals owning their own permits and cages." At no point did the chairman mention profits as a goal or of evidence of success. Also, fish farming is discussed as a project, not as a business or an enterprise. This organization does not yet have a definite business plan, though they anticipate creating one.

The chairman's answers suggests that developing commercial fish farming enterprises is not a goal, but that his members are vulnerable people who want to add a fish farming project to their already long list of development projects. This attitude is reflected in the group members' unwillingness to invest their own financial resources. The chairman says, "There have been no good examples of cage culture in lakes. So the members don't want to invest their money."

The piecemeal approach to aiding vulnerable people seems to manifest itself in members of "The Helping Hands" who are involved in multiple operations to varying degrees, gaining some benefit from each. It is an example of development thinker Robert Chambers' (1997) explanation that, for the poorest of the poor, livelihoods are "local, complex, diverse, dynamic, uncontrollable, or unpredictable." Being a specialized, capital and input intensive, risky, long-term enterprise, commercial cage culture does not fit productively into this type of livelihood strategy.

Uppers and lowers. Chambers' (1997) discussion of "uppers" and "lowers" provides helpful terminology for describing and understanding the relationships of two types of members of "The Helping Hands." "Uppers are people who in a context are dominant or superior to lowers. A person can be an upper in one context and a lower in another" (Chambers 1997 xvi). Conversely, "Lowers are people who in a context are subordinate or inferior. A person can be a lower in one context and an upper in another" (Chambers 1997 xv). There appears to be a strong dichotomy between "upper" and "lower" members of "The Helping Hands". Having the opportunity to spend time with members of both types, evidence of the interactions and expectations of the two groups emerge.

There are members involved in "The Helping Hands" who can be termed "uppers;" they have more education (sometimes holding advanced degrees), their own fish farming operations, or have the resources to become fish farmers (including land, water, ponds, and money). We visited several of their fish farms, including one owned by a physician. These elite members see fish farming as an income-generating enterprise that they manage while hiring someone to provide the day-to-day management of ponds. They also see themselves as aiding members who are "lowers" in gaining income from fish culture. For these "uppers," involvement in "The Helping Hands" organization introduced them to fish farming and provides access to training and some inputs for their fish farming enterprises as well as an opportunity to assist "lowers" in their community.

Several of these "uppers" see a fish farming operation as part of an income-generating farm to which they will retire. One woman, also a physician, stated, "I will do pond culture when I retire. This will be good because I can employ people at home." Her statement demonstrates the dual goals of personal income generation and providing economic options for local "lowers." It also illustrates a conception of fish farming as a sideline activity or a hobby for the wealthy (Moehl 2006).

"Uppers" in "The Helping Hands" are responsible for the management of the fish farms that the "lowers" operate on a day-to-day basis. In this way, "uppers" use their resources to aid "lowers" in the project work and potentially bring the "lowers" out of poverty. The avenues "uppers" use to aid "lowers" is in the procurement of funds for the group's projects, the translation of technical information from English into Lusoga, the local language, and helping "lowers" procure and repay group-sourced credit. The chairman spoke to these relationships when responding to a question about the literacy levels of the members involved in fish farming, saying, "There are those ("uppers") who are capable to help others, to explain in the language that they ("lowers") understand. We are putting the literate at the forefront. A few should manage it ("uppers"). They do this on behalf of others ("lowers")."

Not surprisingly, we had much more interview time with the "uppers" of the group. When conducting interviews with "lowers," "uppers" were always present and sometimes even attempted to guide the "lowers'" responses to questions. This occurred during interviews with the "lowers" who currently manage three very small lakeside ponds and potentially will manage cages on Lake Victoria. These group members live in a markedly poor lakeside community. When I asked why they want to be fish farmers and what they hope to gain from the fish farming enterprise, I received answers such as "The training interested me," and "It is a business enterprise which will bring me money." An "upper," a physician, who will be assisting in managing the cage culture operation, interrupted the "lowers" and answered the question for them: "You get a cross section of people from the local community involved. They will be

able to send their children to school, address the problem of malnutrition, and sell the fish for money. They all show interest and everyone benefits. There are two purposes: to grow food and sell fish for money." The physician attempted to broaden the "lowers" limited, though pragmatic, views of benefits from fish farming to a view reflecting community-development goals. In the process, she silenced them and reinforced her superior social position.

Patronage and paternalism. Further reinforcing the evidence of "uppers" and "lowers" embedded in this group's dynamics is the distinct language of patronage that emerged in this case study alone. The first example is from the conversation between a fishery specialist and the chairman of "The Helping Hands". After hearing that his project would be partially funded, he said, "I am so grateful that Madame (government specialist) has agreed to fund the project. I am grateful in this regard because we are becoming babies of Madame." The uses of the supremely polite title "Madame" and the mother/children metaphor reflect a patronage relationship couched in deference, appreciation, and inferiority.

Later, I observed the chairman in the opposite relationship in a strikingly similar conversation. The chairman of "The Helping Hands" and the middle-aged female chairman of the Uganda Society of the Disabled were speaking together among a group. The Uganda Society of the Disabled is a group that "The Helping Hands"' chairman has aided in establishing pond culture as an income-generating project. The chairman of the Uganda Society of the Disabled said, "I can only thank [the chairman] for his effort. He offered us training and seed stock. I thank him very much. He is a loving father and is caring for us very much." The man previously expressing becoming a "baby" of his own patron, a government fisheries employee, becomes a "father" of the group of disabled people to whom he provides assistance.

Interestingly, in these patron relationships there is no discussion of or question as to the original source of the funds. To the one at the end of the assistance chain, it does not seem to matter if the money came from U.S. taxpayers, a private endowment, or a government agency. What emerges supreme is the deference to the individual immediately passing on financial assistance, reflecting the relational nature of assistance chains (Maranz 2001).

Besides expressing appreciation, applying maternal and paternal vocabulary to relationships of patronage can be understood as a diplomatic, desirous strategy on the part of "lowers," who employ this language to access resources available through patron relationships with uppers (Chambers 1997).

CASE STUDY THREE: "THE FAMILY AFFAIR"

In northern Uganda near the town of Gulu, the center of longtime civil strife is a fish farmer organization that operates a hatchery, produces fingerlings, and maintains a few grow out ponds. This fish farmer association began in 2004, though the chairman has been farming fish on his land since 1973, beginning with a small pond and adding another large pond in 1984. The chairman is a patriarch and is known to his family and his fish farmer association as "Mzee," the Swahili word for "old and wise man."

In 2004, Mzee acted on the local fisheries officer's suggestion to apply to a regional development fund to expand his ponds and build a hatchery. The assistance was specifically designated for farmer groups, not individual farmers. The original fish farmer association formed with 17 people, with 11 males and six females, significantly, all relatives of Mzee. Since then, the fish farmer association has grown to include more than 30 members, including non-relatives. In 2008, the president of Uganda visited the farm and gave money for the construction and management of grow-out ponds, where fingerlings are raised to a marketable size.

Currently, five members own and manage their own ponds in addition to operating "The Family Affair's" farm. Twelve of the fish farmer association's members are Mzee's relatives. The executive members

include Mzee, who has been the chairman since the group's inception in 2004, Mzee's wife, who is the treasurer, a secretary, and five committee members. The group operates several bank accounts to safeguard and segregate money received from the fish farm's operation, donors and other enterprises. Other enterprises include operating an orphanage, beekeeping, and cattle production.

It is an understatement to say that the recent history of northern Uganda has resulted in a population with considerable needs. The challenge of developing commercial fish farmer associations is great. The fisheries value chain manager for an external aid project sums it up, saying, "In the north, people have been receiving handouts for 20 years. It is a difficult pattern to break." However, the linking of prospective producers to their home land can be a positive characteristic of fish farming over enterprises that are not place-based. The secretary of "The Family Affair" PO and an external aid project employee says about the members of the new fish POs, "They are constructing their own ponds so they feel as if they own them." Ownership and land improvement may facilitate these new fish farmers' success. Still, given the recent devastation of this entire region and the obvious physical and emotional needs of its inhabitants, our conversations about business plans, feed conversion ratios, and pond construction seemed surreal and totally irrelevant. The proposition of rebuilding a region that had little in the way of economic and infrastructure resources even before the decades-long reign of civil terror is a formidable one.

Orphan care. "The Family Affair" PO formed in 2004 when violence in the region was raging and many children were in need. Over half of the population of Uganda is under age 15, and only 2.1 percent of Ugandans are over the age of 65 (CIA World Factbook 2010). The chairman speaks of the challenges of that time, saying, "In that time we felt some difficulties to care for the young ones." Mzee's brothers died of HIV/AIDS, leaving him to care for their orphaned children. "Many houses in the community are left with orphans." Two systems simultaneously demand that the chairman cares for his orphaned nieces and nephews: one is a system of traditional responsibility, where the duty of caring for a deceased brother's children falls to brother, and one is an incentive system where receiving donor or government funds depends on performing the role of orphan-caretaker. Mzee says, "We chose to work with orphans because these government structures of assistance require that we reach cross-cutting issues. It is the first step to get the money."

Financial returns from the fish farm's operations are invested into the orphans who receive training in marketable skills, as well as contribute to the farm's operations. "We've paid (school) fees for the orphan children. Some of them are now doctors and teachers," says the chairman's wife. It is unclear whether the fish farm revenues or development assistance received paid the orphans' tuition. Job skills are another benefit the orphans receive. Mzee says, "One of our targets is to get some machines to employ orphans. We can build a workshop. We give them school fees and during the breaks we keep them busy making bricks and training them in that skill." Orphans are also employed to dig fish ponds, an activity that dovetails nicely with the WFP "food for work" approach. This approach requires that the community do the manual labor by digging the ponds, and the WFP supplies the inputs of seed and feeds.

Meetings and records. "The Family Affair's" executive committee meets monthly. The chairman says, "In these meetings we plan, distribute roles, plan for training of other farmers, see what work is done, and see difficulties in the communities within the two districts (Amuru and Gulu). During these meetings the executive committee makes decisions allocating their funds, giving money to the most urgent need, whether that is school fees, fish ponds, feeds, or another need." The entire group of over 30 meets two times per year. Several files are kept by the executive committee and the farm manager, including money received from donors and fish farming operations, fry sales, feeds, and a record of each meeting's events. The chairman comments on the records kept for pond management, saying, "For the feeds file, for example, we record amount of feeds bought, their cost, the source, and quantity daily given to the fish."

Development agencies. One large donor-funded project uses a Farmer Field School (FFS) approach to provide technical assistance. This extension mechanism is an interactive, on-farm learning experience designed to educate farmers, enhancing their ability to make informed decisions concerning their own farm's management (van den Berg 2004).

"The Family Affair" PO will conduct a FFS on every topic of fish production and sale, including value addition, with two members from each PO attending each training session. In addition to educational services that "The Family Affair" PO has been entrusted to provide the groups, the chairman describes the inputs that "The Family Affair" PO will supply to the other POs in kind; "We will help them with money for feed and fry, for every group. For each group we will want to have 3,000 square meters of ponds." "The Family Affair" PO employs extension personnel to provide on-farm advising to the 22 POs.

It is clear that "The Family Affair" PO's activities in developing producer associations and using the farmer field school approach are dictated by donor project goals and requirements. A representative of an external donor project said, "We are trying to look at farmers as our entry point, but not individual farmers. If we worked with individual farmers it would take us 70 years to accomplish our goals. That is why we are looking at farmer groups – we call them producer organizations – of those who are commercially minded and commercially oriented." Commenting on the farmer field school approach, he says, "We bring farmers together for the farmers to identify their own problems and identify solutions together and help link them to other farmers." The "linking" of farmers through "The Family Affair" PO would not have occurred without direction from the donor agency. A Family Affair PO member and donor project technician says, "We are currently working with groups because it is easier for outreach and accessing government assistance."

This service that "The Family Affair" PO provides to the regional POs will prospectively perpetuate "The Family Affair" PO's business model. The secretary said, "We hope to train 600 fish farmers, create demand for our seed, our feeds, and our factory that we hope to build… We need all those we train to become commercial fish farmers so they will come in by themselves and continue to buy feed and fry from us." When the secretary was asked for his assessment of the POs that "The Family Affair" PO is developing, he said, "We believe they will stand on their own after (the large donor-funded project). According to our vision, all the groups will still continue getting fingerlings from us."

The secretary of "The Family Affair" PO is also the project manager employed by a donor project, and he provided insight on previous problems encountered with working with fish farmer groups. "(Pond) management is not done well. There is variation in feeding because many people are feeding." He also speaks of the challenges associated with people transitioning from Internally Displaced Persons (IDP) camps back to their homes, where they attempt to establish farming enterprises, saying, "One of the problems was that some of the groups were formed in the camps where people are together but not necessarily from the same area. So when they leave the camps they are living in distant places. This was a problem in 2007 with the NAADS groups." NAADS, Uganda's National Agricultural Advisory Service, provides financial assistance and training to a spectrum of agricultural producer groups. Also, he sees problems with individuals joining groups without a commitment to fish farming: "All of them should have an interest in fish farming, not just the project."

Goals. When asked about the goals of their producer organization, all executive members interviewed listed construction or infrastructure-based goals that they aim to achieve if donor funding is ascertained. The treasurer, Mzee's wife, cited their need for a water heater for the hatchery, as the solar heater does not supply heat at night. When asked when he hopes to build more ponds, Mzee replied, "You will tell me when you say if you support me." Currently, the hatchery built in 2004 is being renovated through assistance from the external donor project. The chairman stated their three year goal, which is to build a feed mill, and a five year goal, which is to build a fish processing factory for exporting fish to Sudan.

They also anticipate building dormitories and a guest house for those who come to be trained, as well as a structure to house a formulated feed outlet. They would like to build a workshop where the orphans can learn job skills, as well as construct a swimming pool for recreation. Construction of ponds is currently undertaken in anticipation of future donor funds, both for ponds currently under construction and a reservoir. The chairman says, "For us, we keep on making ponds. We are still looking for phase two of NUSAF." NUSAF stands for Northern Uganda Social Action Fund, the regional funding agency that first encouraged "The Family Affair" to form a group.

"The Family Affair" PO's fingerling sales goals are secondary to their infrastructure development goals. This is partially a result of a decreased fingerling market and partially a result of a distorted incentive system inherent in development assistance. Aid programs favor construction projects rather than profitability of enterprises in natural markets.

Fingerling sales. "Between 2004 and 2006 fish farming in northern Uganda had gone down and is now beginning to increase," says a Family Affair PO member and a LEAD-employed fish farming technician. In 2009, "The Family Affair" PO produced 40,000 fingerlings, 30,000 of which were purchased by organizations, including the Food and Agriculture Organization of the United Nations (FAO), AT Uganda Ltd, a national NGO, and the African Development Bank (ADB). Only one producer organization purchased fingerlings from "The Family Affair" PO in 2009.

Since 2004 "The Family Affair's" business structure has been built on accessing donor funds. This requires that "The Family Affair" align their producer organization's goals to the donor's goals. Even the sales of the fingerlings they produce demonstrate the donor saturation in this region of Uganda: 75 percent of "The Family Affair's" fingerlings are sold to aid organizations. Natural markets are not at work here, but given the social and recent-historical context of this region, it may be some time before natural markets emerge as driving economic forces.

CASE STUDY FOUR: "THE COOPERATIVE SOCIETY"

"The Cooperative Society", located in western Uganda, began in 2004 when several members were invited by the minister of fisheries for training at the Fisheries Training Institute (FTI) in Entebbe. The commissioner told them to form groups "in order to be heard and known by government and NGOs." Ten members went for training and upon returning spoke with interested friends and neighbors and began organizing. First, the group registered as an association but changed their registration to a cooperative society at a minister's recommendation. The group is currently registered at all levels, from the local council one, or village level, up to national level, with the Uganda Cooperative Alliance (UCA). This cooperative society is overseen by the head of the Uganda Fish Farmers Cooperative Union and receives technical assistance from the county fisheries officer, who attends gatherings, answers farmers' questions, addresses fish farming problems, and makes farm visits. "The Cooperative Society" also receives some assistance from Uganda Cooperative Alliance and the Ugandan government in the form of fingerlings and training.

"The Cooperative Society's" 90 members include men, women, and youth, with members coming from four sub-counties within the district. Leadership offices are elected positions, and include chairman, vice chairman, treasurer, general secretary, publicist secretary, advisors, and committee members.

Differences between the leaders and members. Two focus group interviews, one with the positional leaders and one with a subset of the members, indicate that there are differences between the members and leaders concerning benefits received from their cooperative society activities and involvement in other types of farming groups and cooperative societies. For example, when asked what other agricultural producer groups they were involved in, the leaders listed beekeeping, dairy production, banana wine processing, organic pineapple, coffee production, poultry production, tree planting, and animal husbandry

as the principle activities of other groups of which they are a part. The members listed poultry production, beekeeping, and banana production, which are agricultural activities that require less up-front capital and with less value-addition components than the leaders' activities.

There are also differences between the leaders and the members of "The Cooperative Society" concerning sources of motivation for joining the group, level of satisfaction with their fish farming enterprises, and extent to which their expectations of the group, the government, and NGOs have been realized. Leaders showed higher levels of satisfaction with their fish farming operations, which is probably related to the fact that leaders had been fish farming longer and had larger fish farming operations than the members, on average. Throughout the discussion leaders' and members' often disparate attitudes are noted. Importantly, leaders were significantly older individuals than the members.

Benefits of membership. One of the primary goals of fish farmer associations is to meet member farmers' technical shortcomings. Therefore, an assessment of farmers' perceived deficiencies in fish culture practice and how these are addressed by fish farmer organizations is a good measure of the viability of a producer organization, especially as it pertains to long-term farmer involvement and growth. Farmers in "The Cooperative Society" identified deficiencies in several areas crucial to their fish farming operations.

First, farmers acknowledged lack of inputs, specifically feed and fingerlings. "The Cooperative Society," through connections with the government and Uganda Cooperative Alliance (UCA), are sometimes given fingerlings for distribution to members. However, these have been given in insufficient quantities or are of low quality and promises of fingerlings are often not met. When farmers purchase their own fingerlings, "The Cooperative Society" also plays a beneficial role by decreasing each farmer's cost through bulk purchase of fingerlings and sharing transportation costs.

Farmers also require fingerlings of high quality, which refers to each fingerling's size, viability after stocking, and subsequent growth rate. In terms of procuring fingerlings of high quality, the collective knowledge, experience, and social capital of the individuals in the producer organization gives farmers access to better fingerling producers and excludes others who peddle poor quality fingerlings. In the same way, the member-farmers who purchase formulated feeds share transportation costs and collectively negotiate for bulk prices. In the future, "The Cooperative Society" aims to serve as a large poultry company's feed vendor for the western regions, which will provide income and further reduce feed costs for members. Member-farmers who are not yet at a scale of operation to purchase formulated feeds receive instruction in making feeds from locally-available ingredients.

Financial shortcomings were at the forefront of member-farmers' stated deficiencies. Many farmers have yet to realize profits from their fish farming operations, though all of them have harvested fish for household consumption. All fish farmers expect profits, and most members who have operated for two production cycles reported generating profits. In addition to teaching productive pond management, the producer organization aids farmer-members in achieving profits through collectively marketing farmers' fish, reducing the time the farmer must spend searching for buyers, as well as reaching the best possible price. Farmers also receive advice on marketing and pricing their fish.

Farmers with a desire to expand their fish farming operations find access to capital to be a problem, especially in terms of credit and land; lack of capital is often an inhibiting factor in improving their fish farm's productivity. The producer organization, while not currently aiding farmers in accessing credit, hopes to increase resources to the point of providing production-cycle loans to member farmers.

One way that "The Cooperative Society" acts as a financial safety net is through an emergency fund that it maintains for its members. Farmers annually pay into this revolving fund and are able to access small

loans to pay unexpected bills unrelated to fish farm operations, such as a death in the family or hospital bills. In this way, "The Cooperative Society" also functions as a burial society, one of many such societies to which farmer-members may belong. Burial societies serve an important function in terms of civil society and financial security (Makumbe 2002). Thus the cooperative provides broader social and economic benefits to its members beyond inputs and guidance for fish farming.

In fish farming training, farmers were eager to learn environmental improvement techniques that they integrated into their fish farming operations. They mentioned water harvesting and decreasing erosion through pond side tree planting as conservation efforts they employ. Leaders in "The Cooperative Society" identified human capital-enhancing skills they developed while occupying elected positions. These included skills in business, leadership, communication, English, marketing, learning from one another in the group, hearing new ideas from outsiders, and growing in personal confidence.

Fish farming as status symbol. A common benefit cited both the leaders and members of "The Cooperative Society" derived from their fish farming enterprises as well as through leadership positions they held in "The Cooperative Society" the status in the community. Farmers take great pride in their fish farming enterprises. This pride is reflected in the physical care and management of ponds, evidenced by the well-kept grass, as well as the ways the farmers use their fish. The act of a farmer serving fish he or she had raised at a special event, such as a child returning home from boarding school, or to important people, like visitors, is both a demonstration of achievement and status and a source of farmer pride.

A special meal is usually served to children returning from boarding school and fish farmers who are able to serve fish are offering their children a treat: "Fish is something they never would have eaten at school." Also, fish farmers discussed how their fish ponds improved the appearance of their homes. Ponds demonstrate the ability to develop their resources and this physical evidence increases their neighbors' perception of the farmers' success. One fish farmer said, "A neat and well-organized home is a symbol of status."

The ability for fish farming households to feed fish to their families is also a source of pride as they actively provide nutritious, high-value foods for their children. Farmers who were receiving income from their ponds spoke of the increased prestige that their improved incomes brought as well as the ways they invested this income into land and education. One farmer mentioned expanding his land holdings as a result of fish-based income. Several spoke of the pride they felt from sending their children to boarding school with income from their ponds. Finally, farmers were proud to be able to share fish harvests with their disadvantaged neighbors, knowing that they had a nutritious, valuable food to offer. While farmers cited compassion and empathy as reasons for gifts of food to poor neighbors, sharing fish is also an important demonstration of agency and wealth.

Leadership. Discussions with the leaders revealed the status conferred on elected cooperative society leaders. Being elected to a position in a society is public recognition of status and affords opportunities to further improve status. Fish farmers holding leadership positions in "The Cooperative Society" talked about the business and communication skills they had gained through their roles. One man who had limited schooling was able to improve his English through interchanges with more educated peers. Also, leaders are often nominated to go to training and bring back the information they received to share with the members. The opportunity of learning information first and presenting it to members at a meeting reinforces the leaders' status.

Several leaders are retired. In Uganda, government employees are required to retire at age 60. After retirement, their community involvement and status usually decreases. Involvement in "The Cooperative Society" is a means of maintaining their community-serving and active lifestyle. One woman, a retired teacher and committee member who proudly pointed out her former students among the members, shared

the confidence and influence she maintains post-retirement through her involvement in this organization. She holds a leadership position and therefore a responsibility to be busy and engaged. She says, "I am able to pick up my nice dress, put it on, and I forget my old age."

Leaders articulated several key areas where networking and advocating for the fish farming sector are important responsibilities of their producer organization. Consistent with the society's goal of addressing farmer deficiencies, the leaders seek to "Work together to solve the challenges of fish farmers with one voice." In order to unite the fish farmers' voices the leaders have sought out relationships with fish farmers outside their producer organization and thus built social capital. The president boasted, "Now we know all the fish farmers in the entire county."

The leaders interact with individuals and groups who have resources that their member famers need. These resources include fingerlings and training and are sought through relationships with government officials, foreign donors, and the UCA. With an understanding of the linkages between fish farming and other development arenas, the leaders have aligned their fish farming goals with goals such as poverty alleviation, environmental preservation, and malnutrition, especially as it is experienced by HIV/AIDS victims. Advocating for the fish farming sector includes recruiting new fish farmers, and "… spreading the message that households with land and water can earn good incomes through fish farming." Thus the logic and objectives of the donor shape the direction of the cooperative.

The Cooperative Society leaders actively plan to expand its presence as a locus of fish farming specialization. They state that the society's success is built on the member-farmers' success, which explains why their first goal is to increase all members' fish production and thus, household income. For some, increases in income from fish farming have already lead to sums sufficient to purchase more land to expand fish farming operations and pay children's school fees. Plans to rent an office space, sell formulated feeds, and offer production-cycle loans to members are all part of their vision to increase member-farmers,' and therefore "The Cooperative Society's," success. Leaders also articulated several community-development goals, such as creating opportunities for local youth with little education to earn incomes from pond construction and a fish consumption goal for the community to which they belong. One leader cited the FAO nutritional recommendation that individuals eat fifteen kilograms of fish per year, and her vision is for the fish farmers in "The Cooperative Society" to supply that amount of fish for local consumption.

PART II: CAGE CULTURE TRIALS

Following group member meetings and training on stocking and management of the cages discussed above, the group was set out to grow their first lot of fish. The results presented below are based on the data collected up to day 84 following stocking of the cages. This is the last time sampling was carried out. The results thereafter are a projection based on the previous sampling.

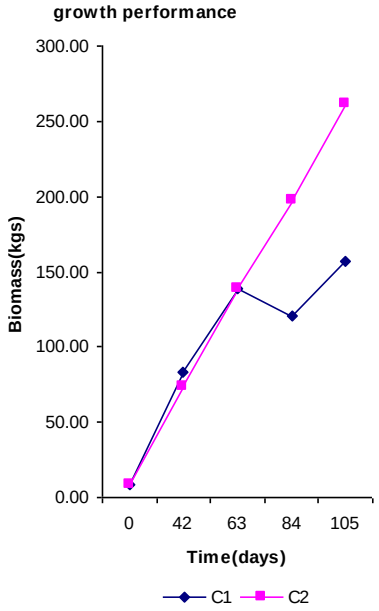


Figure 1. Growth performance
The graph shows growth performance of cage 1 (C1) and Cage 2 (C2). The biomass of cage 1 and 2 increased exponentially for the first 2 weeks but later the biomass of cage 1 decreased on day 84 and eventually increased on day 105.

The significant drop of biomass in cage 2 was due to fish that escaped through holes that were discovered on the net bag. However, the biomass of cage 2 increased at a relatively constant rate reflecting a steady fast growth up to day 105. This was an indication of normal growth.

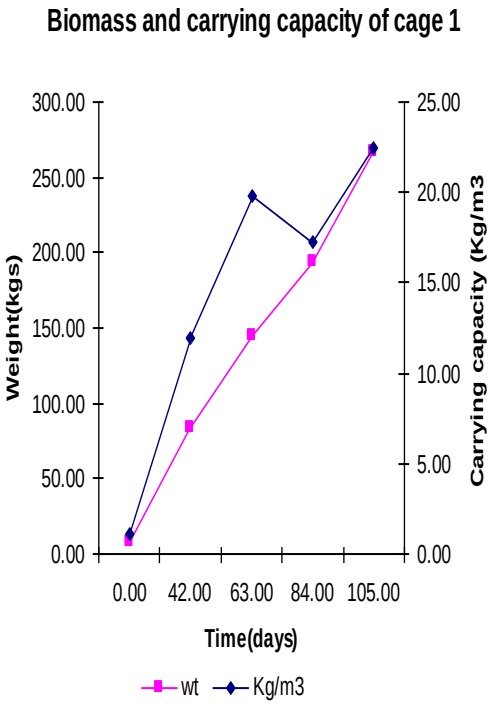


Figure 2.

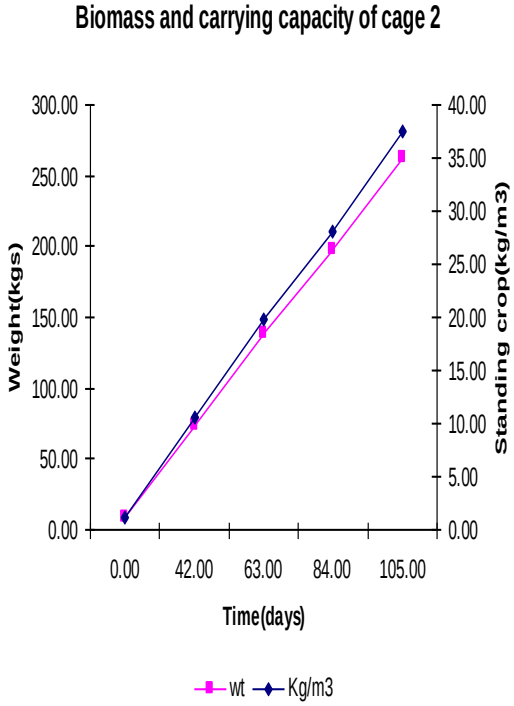


Figure 3.

The figures above compare the biomass of cages 1&2. Figure 2 shows an exponential increase in the biomass of cage1 together with its carrying capacity. However the biomass on day 84 decreased as well as its carrying capacity. This was because when fish escaped there was a reduction in numbers, total weight and the carrying capacity per $m^{3.}$

Figure 4 shows biomass and carrying capacity of cage 2 increasing at almost the same rate for a period 105 days. This was because this cage experienced low mortalities and no fish escaped hence maintaining a favorable stocking density.

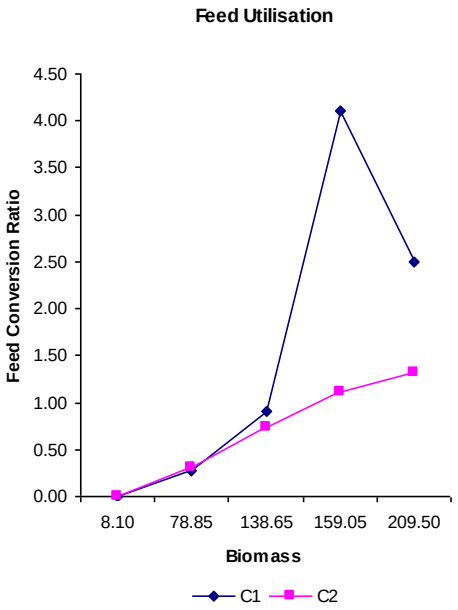


Figure 4. Feed utilization: The graph shows utilization of feed by cage 1 & 2 with increasing average biomass. At average biomass 78.85 the FCR of cage 1 & 2 is less than 0.5 because the fish supplement their diet with natural food which is mostly phytoplankton.

Cage 2 showed a steady increase in FCR together with increasing biomass from 78.85 to 209.5 kg. This indicated that almost all feed eaten by the fish was converted into body weight. This rendered them more efficient at digestion and utilization of feed as compared to cage 1.
However, cage 1 shows a sharp increase in FCR with increasing biomass from 78.85 to 159.05 kg. This could have been due to fish escape to the wild. As a result, most of the feed administered during this period was in excess and wasted to the lake as uneaten feed.

These graphs emphasize the importance of sampling as a key aspect in monitoring current fish biomass. This enhances determination of right quantities of feed that should be administered hence minimizing losses due to applying excess feed.

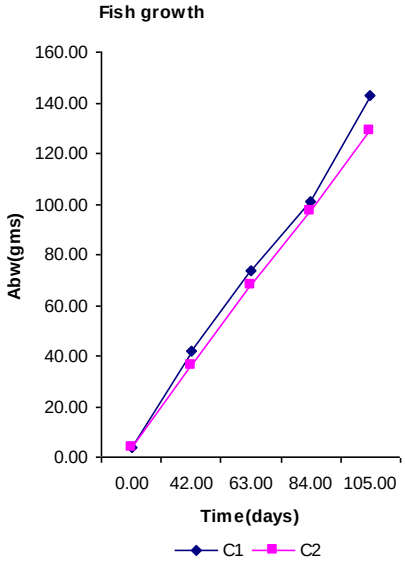


Figure 5. Average body weight with time: The graph above compares size of fish in cage 1 & 2. Initially the growth of these two cages is almost the same. However, day 84 shows cage 2 with a faster growth than 1. The faster growth was due to lower stocking density in cage 1 due to fish escapes. This resulted in less competition for available resources like food. As a result growth was accelerated in cage 1 than 2.

The average weight attained in both cages within 84 days is about 100g. This size is more than most of the undersize capture fish sold at an informal landing site at Kirinya, an illegal fish business between fishers and wives of prison warders[2].

Small holder farmer group organization. Jinja United Group Initiative for Poverty Alleviation and Economic Development (JUGIPAED) secured most of the key requirements (water quality parameters, permit, site, market information), to start a project on cage fish farming on Lake Victoria. Later, the group entered into partnership with the Aquaculture Research and Development Centre, Kajjansi with funding from AquaFish CRSP to carry out cage fish farming at Kirinya. The partnership is on a cost sharing basis and it is intended to provide a kick start to implementation of cage culture project agreed upon by the group members while at the same time carrying out research in cage culture. The rationale for this approach is demonstration of fish farming as a business, hence the need for the group to contribute to the costs of the enterprise and learn how to manage their cash flow.

Stutzman (2010) observes that aquaculture development commentary supports the formation of fish farmer associations or producer organizations as avenues for cultivating small- and medium-scale commercial farmers. Umbrella organizations under which local fish farmer associations vertically align themselves have important implications for fish farmer production. Formation of small holder farmer group organizations has been encouraged by government of Uganda in order to ease provision of various services particularly technical advice and inputs such as seed. Some of these farmer organizations have initiated self-help activities carried out as a group most notably savings and credit. When JUGIPAED decided to engage in cage culture, the members used their collective savings in the group's account to cover their share of items that were agreed upon as per the MoU. These items included feed, labor, and any other expenses such as communication and transport costs to the site.

However, as earlier noted by Stutzman (2010), group cohesion and participation by all group members seem to be a challenge. This stems from the fact that groups tend to front numbers in order to attract support from government or donors, hence actual group activates are often engaged in by just a few members and not all listed in the group's register. However on the other hand, group managed activities are in themselves a challenge especially if there are uncertainties on issues such as the potential risks involved and benefit sharing. Consequently only a few members keep the work moving.

Cash flow management. A draft enterprise budget to guide the trial was drawn by the project team and discussed with the group members. During the discussions, inputs to be contributed by either party were agreed upon and included in the MoU. During the trial, the chairman and treasurer of the group kept all the records pertaining to cash flow. The records showed sources of funds which are mainly members' contributions. The project team also availed group members with information on cost of items contributed by the project. Discussions were held with group members on the variable costs incurred for cage operations up to day 84.

[2] The business emerged shortly after the cages had been stocked.

Table 2. Operating costs of managing the cages for 3 months

Input description	Unit cost (UGX)	Total cost (UGX)
2030 fry	80	162,400
132 kg of feed	2167	286000
Labor for feeding per month	50,000	150,000
Transport by members	8,000	60,000
Total		658,400

Basing on UGX400 which is the average price of the 80-100g tilapia fish sold at Kirinya, we estimated that the anticipated revenue from the 209.5kg from cage 2 to have been UGX 836,000 culminating in a profit of UGX177,600. However, if the fish were to be sold by a kilo at UGX 3,500, the revenue would have been UGX731,500 making a profit of UGX73,100. This indicates that it is profitable to sell fish at the smallest market size as long as positive returns above variable costs can be attained.

CONCLUSION

Across cases, several similarities emerge. Each fish farmer association operates in an area of high potential for aquaculture in Uganda. Fish farmer associations are place-based, with members from a defined geographical region. Each operates in an umbrella group structure. That is, each fish farmer association has other farmer associations "under" it or has an organizational structure "over" it. Also, no full-time fish farmers emerged from the groups examined; all group members and leaders stated that they are involved in other agricultural producer groups, with many individuals involved in three or more agricultural producer groups. For only one fish farmer association, "The Family Affair", is fish farming the primary economic enterprise for executive members, and even this fish farmer association is involved in other agricultural activities.

The thread of misdirected development assistance runs through each of the following categories of discussion. It should go without saying that the primary goal of a fish-productive aquaculture producer organization cannot be orchestrating its activities to qualify for the most donor assistance possible. Nonetheless, there are multiple aspects at play in the relationships between each of the fish farmer associations examined and funding agencies (both governmental and NGO). These relationships are considered in light of the ways the structures they produce aid or inhibit fish farmer associations in strengthening profitable, commercial member farmers.

Specifically, across cases, the catalyst for group formation influenced each producer organization's goals and priorities, as well as members' expectations. Members' expectations are shaped by the promises of the government official encouraging the individuals to form a fish farmer association. Also, catalysts for group formation and subsequent priorities and goals are directly related to members' fish production. Fish farmer association goals and priorities determine whether or not the member farmers and leaders view their activities and enterprises as successful. In instances where the goal of engaging in fish culture is to receive money rather than generate income, success is not measured in fish production, but in the amount of money received (Grivetti 1982).

Across cases, every producer organization formed based on the advice or encouragement of government officials and group formation was related to receiving funding for the producer organization's activities. Though no case besides "The Family Affair" kept concrete production records for their organization, based on farmers' assessments of production and profitability, some conclusions can be drawn about the connection between donor support and fish production or fish farm profitability.

"The Unaccountable Leaders" worked through an existing community based organization (CBO), an association dedicated to environmental conservation, in order to receive government support for their fish farming activities. However, there is no system or mechanism for equitable distribution of benefits among members of this group-managed fish farm, even though much of the funding comes from government agencies or donors. The fish farming project coordinator says, "People who have put in big investments must take the lion's share," implying that the project coordinator himself, who arranged for the funding, was the "lion."

"The Helping Hands" producer organization was made up of a subset of members of a regional organization focused on poverty alleviation. When the chairman was asked why this organization was formed, he replied, "The idea was to serve the needs of the members of the group and to get creditors." This group works with cross-cutting issues, in response to donor goals; in order to receive funding from NAADS, the group must provide HIV/AIDS education to its members. This producer organization has received or sought funds from donor-funded projects, as well as local government agencies. Because this organization has not begun cage farming no assessments can be made about fish production.

"The Family Affair" was a functioning fish farm for 30 years, from 1973-2004, and operated by an individual and his family, until a district fisheries officer advised the farmer to organize as a group in order to be eligible for regional, government-sourced funding. Still, many members of this producer organization are the chairman's family. Besides accessing funding based on having a group structure, the name of the association includes the word "orphan," which expands the chairman's entitlement to donor funds. The chairman's brothers died of AIDS, leaving him with the responsibility of providing for his nieces and nephews. When asked about the organization's connection to orphans, the chairman said, "We choose to work with orphans because these government structures of assistance require that we reach cross-cutting issues. It is the first step to get the money." This producer organization has received funds from a regional funding agency, WFP, and USAID.

"The Cooperative Society" began as an association, but the leaders changed their organization's registration after the minister of fisheries advised them to form a cooperative society. This registration change allowed them to receive assistance (or, the promise of assistance, as many promises have not been fulfilled) from the Uganda Cooperative Alliance (UCA).

Each producer organization operated within a larger umbrella structure, where fish farmer associations are affiliated with a larger organization: "The Unaccountable Leaders" producer organization is under a regional association dedicated to conserving environmental resources; "The Helping Hands" is a sub-set of members of a poverty alleviation organization who share the goal of cage culture, as well as a regional administration and funding structure of fish farmer groups throughout the region; "The Family Affair", at the mandate and expense of external donors, is overseeing the development of 22 other fish producer organizations s; and "The Cooperative Society" is a regional producer organization under the umbrella of the Uganda Fish Farmers Cooperative Union, and also registered with the Uganda Cooperative Alliance. The impacts of these "groups within groups" structures require further study, though some important elements emerge from our research.

From the four cases examined, the most significant impact of the umbrella structures was that the goals of the "umbrella" organization color the goals of the groups they "cover." When this "cover" is tied to financial support, the goals become mandates. Often, the goals of the funding agency do not include developing commercial fish farmers, though this may be a primary goal of the producer organization.

Funding agencies' directions can potentially distract producer organizations from their objective of developing productive fish farmers or promote strategies that are ineffective in practice. Part of the reason for this promotion is that fish farming is touted by government officials as a profitable farming enterprise

that anyone can do. The perception is: men and women, able-bodied and disabled, wealthy and poor, widows and orphans, everyone can earn money from fish farming. While most successful fish farmers and technical experts seriously question the validity of that perception, government officials still design and fund projects to organize fish farming projects connected with reaching unrelated goals. Examples of funding agency goals unrelated to productive fish farmer development include reaching cross-cutting issues such as providing HIV/AIDS education and reaching vulnerable populations (i.e. women, orphans, and disabled people). An example demonstrates the ineffective strategies of one of these efforts: a fish farmer group made up of disabled people operating under "The Helping Hands" producer organization cited problems with physical mobility as one of their major constraints to operating a profitable fish pond. Their mobility-related disabilities prevented this group from efficiently managing their ponds. According to their production records, the group of disabled people found fish farming financially unsustainable and plans to abandon production.

However, fish farmers' ability to improve the lives of the poor is not only accomplished through training vulnerable people as fish farmers, and may not require funding agency dictates. The producer organization with the least donor support, "The Cooperative Society", addressed cross-cutting issues quite differently than "The Helping Hands" or "The Family Affair", the two most donor-involved producer organizations. "The Cooperative Society" members aided vulnerable people as individual farmers, not as a collectivity, by providing poor neighbors with on-farm employment opportunities and sharing nutritious, farm-raised fish.

In the cases examined the umbrella structures that specialize in fish farming yield member fish farmer associations with higher production than umbrella structures that oversee a spectrum of projects. "The Cooperative Society," under the umbrella of the Uganda Fish Farmers Cooperative Alliance, and "The Family Affair," are the two highest-producing fish farmer associations examined.

Fish production-based umbrella structures are better able to develop productive fish farmers partially because of the social capital these associations develop: bonding social capital, which unites the members of a producer organization and bridging social capital, which connects people and institutions. A host of relationships set these specialists associations apart, as they have long-term working connections with technical experts, government research stations, universities, international experts, fingerling producers, feed distributors, and development professionals. Through these relationships, fish production-based umbrella structures are better poised to advocate for the fish farming sector, broaden member farmers' resources, and develop productive fish farmers.

Additionally, umbrella structures which specialize in fish producer organization development are less likely to seek funding for non-aquaculture related development projects, efforts which distract diversified umbrella associations from focusing on improving fish farmers' successes.

Several incentive systems designed to encourage the development of a profitable and commercial fish farming sector in Uganda have been distorted to the point that they inhibit the economic and human-capital growth they were conceived to foster. What were designed to be incentives to productive fish farm development have evolved into ends in themselves. When leaders profit from distorted incentive systems, members' trust is seriously compromised and member attrition results.

Two leaders of producer associations expressed that they wanted to operate model farms. The leaders of both "The Unaccountable Leaders" and "The Helping Hands" expressed this interest. Also, these two men are most politically ambitious and donor-seeking PO leaders. In Uganda, a model farm is a political distinction. Rather than recognizing farmers who have built up productive and economically successful farm enterprises through the farmer's own long-term investment and expertise, model farms can be designated before one complete production cycle. In this context, a model farm is one that has been

recognized by the president and designated as a demonstration farm for farmer field school education. With model farm distinction comes an inflow of government assistance. This system is well suited to limited funds and staff members but, as previously mentioned, ordinary farmers may perceive model farmers as a privileged group they are unable to mirror (Mangheni 2007). This understanding limits the application of information received during farmer field schools held on model farms. Both of the producer organization leaders interested in achieving model farm status are envisioning the rewards, in terms of money and influence, which are unrelated to fish farm profitability. Yet the rewards from donor money are often more tangible and immediate than proceeds from fish culture. Model farm distinction is a financial end in itself; it is tangentially related to farm commercialization.

The reality of producer associations maintaining multiple bank accounts for categories of donor assistance offers an insight into a pattern of assistance-seeking. Related to the treadmill of development assistance, many producer organization leaders pursue a piecemeal approach to funding sources.

This approach is borne out of the development paradigm of cost sharing, where assistance-receivers invest a percentage of their own financial resources into a project. The purpose of cost sharing is to encourage participant ownership of the project and thus, incentive to manage the project well, as to provide returns on the participant's investment. Since a producer organization leader realizes that development agencies expect cost sharing, he pursues multiple donors. For example, if one donor will finance 80 percent of a project, and the group members are expected to contribute 20 percent of their own financial resources, the producer organization leader may not ask his members for the 20 percent but finds another donor, unbeknownst to the first, to finance the 20 percent that is the members' responsibility.

If the leader is also a local politician, or has political aspirations, this piecemeal approach becomes even more important, as the leader will lose popular support if his or her participants invest their own resources into a project that fails. With membership dues or participant investment come expectations of leaders' accountability and financial returns. In the words of the project coordinator of "The Unaccountable Leaders'" producer organization, "We have people who are ready to pay money to be members but we are not signing them up because we can't take their money when there are no feeds because then they will be asking, 'What is happening with our money?'"

To clarify, this is not a greedy or underhanded approach to conducting business but a practical one. This approach was created (and is sustained) by the revolving door of donors and government programs designed to assist the poor farmers of Uganda. A half-century's history has proven that in time, another donor will come; therefore investing personal financial resources is unwarranted, if not wasteful. However, the piecemeal approach to funding sources has a detrimental impact on the aquaculture development of Uganda as it perpetuates the idea that fish farming is only profitable if a donor pays for the fingerlings and feed.

Though patterns of distorted incentive systems and piecemeal donor seeking were established by donor behavior, the effects damage the viability of fish farmer associations and undermine their ability to accomplish the goal of becoming profitable commercial fish farmers. As previously mentioned, with each donor comes that donor's own aims, which may or may not align with the producer organization's goals. In fact, government or donor goals may serve to hinder member fish farmers from focusing on production, profitability, and long-term organizational viability. Donor and governments' requirements certainly threaten fish producer organization leadership development, as this pattern of goal displacement and distortion obstructs leaders from defining, working towards, and achieving goals and forming an organizational identity.

In the current method of operations, leaders of donor-driven fish producer associations simply follow the dictates of donor organizations, dictates which change with the creation and completion of an endless

stream of short-term projects conducted by an alphabet soup of donor organizations. Additionally, fish producer organizations model the donor's short term project orientation. For fish producer organizations in Uganda to support a market-driven, thriving aquaculture sector sustained over time, producer organization leaders must recognize that current government and donor financial incentives are not serving their interests as commercializing fish farmers, and avoid them while demanding that these structures be reformed to serve the intended purposes of governments, donors, and fish farmers.

LITERATURE CITED

Brummett, R. E. 2008. African aquaculture: Realizing the potential. Food Policy, 33, 371-385.

Chambers, R. 1997. Whose Reality Counts? Putting the First Last, Practical Action Publishing, Bourton on Dunsmore.

de Seligny, P. J.-F., A. Gumy, R. Grainger, U. Wijkstrom and T. Farmer 2006. The state of world fisheries and aquaculture. in F. F. a. A. Department editor. Food and Agriculture Organization of the United Nations, Rome.

Grivetti, L. E. 1982. The Rationale, Promise, and Realities of Aquaculture: A Cultural-Nutritional Perspective. in L. J. Smith, Susan Peterson editor. Aquaculture Development in Less Developed Countries: Social, Economic, and Political Problems. Westview Press, Boulder, Colorado.

Harrison, E. 1996. Digging Fish Ponds: Perspectives on Motivation in Luapula Province, Zambia. Human Organization, 55, 270-278.

Hecht, T., J. Moehl, M. Halwart and R. Subasinghe 2005. Regional review on aquaculture development 4. Sub-Saharan Africa -- 2005. FAO, Rome.

Hennink, M. M. 2007

International focus group research: A handbook for the health and social sciences, Cambridge University Press, Cambridge.

Makumbe, J. M. 2002. Is there a civil society in Africa? International Affairs, 74, 305 - 319.

Mangheni, M. N., editor. 2007. Experiences, Innovations and Issues in Agricultural Extension in Uganda: Lessons and Prospects, Fountain Publishers, Kampala.

Maranz, D. E. 2001. African Friends and Money Matters, SIL International Dallas.

Moehl, J., R. Brummett, M. K. Moniface and A. Coche 2006. Guiding principles for promoting aquaculture in Africa: benchmarks for sustainable development. FAO, Accra.

Molnar, J. J., N. B. Schwartz and L. L. Lovshin 1985. Integrated aquacultural development: Sociological issues in the cooperative management of community fishponds. Sociologica Ruralis, 25, 60-80.

Mosher, A. T. 1966. Getting agriculture moving: Essentials for development and modernization, Frederick A. Praeger, Publishers, New York.

Mwanja, W. W. 2005. National Aquaculture Sector Overview: Uganda. in F. F. a. A. Department editor. FAO, Rome.

Stake, R. E. 2006. Multiple Case Study Analysis, The Guilford Press, New York.

van den Berg, H. 2004. IPM Farmer Field Schools: A Synthesis of 25 Impact Evaluations. Global IPM Facility. Wageningen University, Amsterdam.

Yin, R. K. 2008. Case study research: Design and methods, SAGE Publications, Thousand Oaks.

Training and Outreach in Uganda and Surrounding Nations

Production System Design and Best Management Alternatives/Activity/09BMA02AU

Joseph J. Molnar and Karen Veverica
Auburn University
Auburn, Alabama, USA

Nelly Isyagi and Maurice Ssebisubi
Aquaculture Management Consultants Ltd.
Kampala, Uganda

Monica Karuhanga Beraho
Makerere University
Kampala, Uganda

INTRODUCTION

Research, extension and education can contribute greatly to enhancing aquacultural production in a sustainable way and to reducing poverty, but achievements have generally fallen short of expectations in Africa (Sanginga et al. 2008). Farmers trust the experience and knowledge of others who are in situations similar to their own. Their desire to meet and talk with each other has spurred the formation of groups and networks to foster informal gatherings and more formal mechanisms of association to facilitate peer-to-peer learning. Such learning groups are most effective when they have a targeted membership like fish farmers. If member perspectives are too diverse, then participants tend to become disenchanted because the results do not apply to their situations (Barrett and Ewert 1998).

Peer-to-peer learning and support systems become increasing important in the context of privatized extension (Klerkx and Leeuwis. 2009), but in Africa there is often little or no reliable extension system to privatize. External donors endeavor to foster private, non-profit mechanisms that will be sustainable and provide the information and organizational services that fish farmers need to build an industry.

Farmer innovators appreciate exchange and study visits as ways of gaining new experience, knowledge and techniques, which they informally experiment on at home (van den Ban and Hawkins 1998). Farmer-to-farmer communication is more effective when visitors and hosts are well prepared, and if both groups review the usefulness of the exchange and deliberate on the reporting of lessons learned.

Previous experience with farmer innovators in agricultural development suggests that study tours and farmer-to-farmer interaction led to significant levels of advancement in production practice. The project encouraged innovators to organize themselves into clusters of farmers and exchange experiences within and between clusters. In Tanzania, some farmer innovators started forming local groups with neighboring farmers after returning from the exchange visits. It is not easy to fully integrate the farmer innovation approach to participatory research and extension into the regular activities of national institutions. The concept of farmers as innovators and researchers is still new for many decision-makers. Thus, there are manifold ways that innovator farmers that share a common interest in a focal enterprise such as fish culture will associate themselves to gain the benefits of mutual support and collaboration.

Outreach is extension, and implies regular and purposeful communication with stakeholders and beneficiaries at the various intermediate and local levels (Kerrison 2005). When standard forms of

literacy-based and electronic communication are no longer available, then outreach takes place in the traditional extension way, with physical visits, dialogues, community meetings etc. Where adequate mass communications, electronic or literacy-based media can be used, mobile phones, email etc. then the need for travel and face-to-face meetings is obviously reduced (Kerrison 2005). The results (and other information) are disseminated through a series of study tours and fish farmer symposiums that involve selected fish farmers from neighboring IEHA countries. We will organize and carry out several training events in Uganda.

ANNUAL FISH FARMER SYMPOSIUM AND TRADE SHOW

The annual fish farmers' symposium and trade show has drawn participants from around the country and the region. The project will participate in organizing these symposiums by helping build the program and in some cases participating as speakers or resource persons. These events also provide a forum from which to disseminate the activities of the AquaFish CRSP. During the trials and particularly for the first cage harvested at each site, a site visit will be hosted by a fish farmers' group that will present their findings and experiences. The annual fish farmers symposium and trade show begun in Uganda in 2007 under the USAID funded Fisheries Investment for Sustainable Harvest (FISH) project. The project was small and operated in a focal pilot area of Central Uganda around Kampala operating in Iganga, Mukono, Wakiso Mpigi and Mityana Districts. Farmers and service providers around the country raised concern about the fact that they were unable to access information from the project, though the demonstration farms and training sessions on these farms were open to all.

Among their concerns, was while they could come to these sessions, the costs of travel several times a year for a specific topic was costly/not cost effective for them given their finances and impact on production when they were not on farm/the effect of them spending too much time away from their farms. They would rather a session was organised for them over a day or two where all information and finding for that year were communicated to them, questions asked. Holding a symposium seen to be the most viable option. Thus the first fish farmers' symposium where the first results of the FISH projects were disseminated to stakeholders in the fish farming across the country. The trade fair to demonstrate technologies and link farmers to suppliers of inputs for these technologies and sources of more information.

The level of interest was high from farmers and service providers. And at the evaluation of the first symposium it was observed that having more farmers from other parts of the country also present would enable direct discussion and answer questions. At second symposium WAFICOS offered to play a more active role realising the benefits and that one of their objectives was to disseminate quality and vetted technical advice to their members as many farmers where victim of poor advice. They realised it was a place for obtaining good advice, a farmer lead forum where farmers could openly air their views and concerns, and not a technocrat lead event where farmers are often relegated to being passive participants. At the second symposium, WAFICOS undertook to conduct these annually however, was limited in resources. However, they realised that there was more than meets the eye regarding the organisation of the symposia. (which the CRSP project covered)

Specifically, the purpose of the symposia and trade fairs are to:

1. To share farmers experiences in overcoming constraints in fish farming practice in the transition from subsistence to viable market oriented commercial enterprises.
2. To share the experiences of industry and other service providers of investing in and meeting the needs of a new emerging sector.
3. To exchange information on status and local innovations to overcome challenges faced by farmers and those involved in the aquaculture value-chain.

4. To promote collaboration among stakeholders in the aquaculture sector to enhance sustainable development.

I.

OBJECTIVES

1. Conduct an annual Farmer to Farmer study tour for producers from Kenya and Tanzania.
2. Organize Annual Fish Farmers' Symposium and Trade Show to disseminate project research results, provide reliable technical information, and share producer perspectives on fish farming industry trends and conditions.

II.

METHODS AND MATERIALS

Conduct Farmer to Farmer Study Tour for Producers from Kenya and Tanzania

The study tours organised for farmers from the neighbouring countries were linked to the Annual Fish Farmers Symposium and Trade fair as it was felt that the information and exchange of ideas with the wider sector of farmers/service providers at these venue would be beneficial to them. Thus, the study tours were organised for the day after the symposia and invitations sent out.

Organisation of the Fish Farmers Symposium and Trade Show

The farmers through WAFICOS took the lead role in the organisation of the event and identification of topics for the event. They solicited additional funding for the event, identified presenters, and approached participants for the trade fair. CRSP through the host PI and Aquaculture Management Consultants liaised with WAFIOCS to identify key areas, speaker's issues.

The support offered included reviewing and discussing topic lists and issues, identifying what sort of data would be required to bring out issues and illustrate key points the farmers wanted brought out or information on, collecting and analysing this/farmers' data as not all farmers were able to do this themselves, compiling presentations, verification of information and sources, compiling and editing symposium, records of attendance, preparation and multiplication of hand-outs during the symposia including proceedings of previous symposia., making of CDS, analysing evaluation data and making evaluation reports, loading the information onto the internet. Arranging the study tour and logistics for the study tours. WAFICOS members and other farmers were made open to Uganda farmers. The previous symposia did not have study tour attached. Advertising, Aquaculture Management Consultants Limited made the websites for the symposia both for the announcements and of the proceedings. Proceedings then given to agencies that supported, and other stakeholders that included the government institutions and private sector.

An optional one-day field tour to various aquaculture-related establishments was organized whose major objective was to expose farmers to successful aquaculture operations and establish contacts with input suppliers and vetted service providers (see Appendix 2).

Other Seminars and Tours

The outreach component collaborated with the other study teams of the Uganda project to organise seminars on specific aspects.

RESULTS

Conduct Farmer to Farmer Study Tour for Producers from Kenya and Tanzania

Farmers from Kenya were invited to the 2010 and 2011 annual symposia. The CRSP Kenya coordinator was instrumental in passing on the invitations. However, it was at the 2011 symposium that Kenyan farmers responded. Two attended and appreciated the benefits of the symposium and accompanying study

tour. On their return they passed word round and a special study tour was organised for a group of ten farmers and extension personnel.
The farmers were asked their interests and the tour was tailor made to their needs. A special hand-out was made of the places they were to visit by Aquaculture Management Consultants Limited. WAFCIOS collaborated by linking up with farmers and accompanying the farmers around (their program coordinator, Ben Kiddu).

Organisation of the Fish Farmers Symposium and Trade Show
The 2010 and 2011 symposia were undertaken with the support of CRSP. A combined total of about 300 participants attended and 34 presentations were made. Of these 4 of the presentations presented CRSP results and they were in line of the farmers' themes for that year. Most of the presentations were done by farmers regarding their experience.

The 2010 Symposium: The theme of the Third Annual Fish Farmers Symposium and Trade Fair in 2010 was "**Dealing with the Challenges of Building an Aquaculture Industry**". This arose largely because during the year 2009, there were significant shortfalls in the supply of key inputs, notably feed and seed, yet farmers had increased their levels of investment into fish farming in view of the good performance they had obtained in 2007/08 with the formulated commercial feeds from Ugachick Poultry Breeders Limited and Source of Nile Limited and a general improvement in the quality of seed produced by local hatcheries. Levels of production and sales rose during that period.

Thus 2009 was a difficult year as it was too late for farmers to pull out considering the fish in stocked units and additional number of ponds that had been constructed. The only alternative was to make it work to salvage their investments as particularly the feed supply situation was temporal while Ugachick was upgrading it fish feed producing facilities to produce floating fish feed.

The focal points of discussion at this symposium were:
1. Assessing the key production factors affecting the viability of fish farming enterprises.
2. Identify and review challenges faced in accessing inputs and the implications on returns to investment and quality of service delivery.
3. Assess the availability of potential markets, and market information for fish farming.
4. Review the availability and quality of current support services to the aquaculture private-sector and factors affecting their accessibility.

Papers Presented at the 2010 Symposium: Table 1 lists the papers presented at the 2010 symposium. The key points for discussion were water supply for production, pond construction, feed and seed availability, accessing finance and grants. The symposium runs for two days

2010 Trade Fair: There were 12 exhibitors at the trade fair that run concurrently with the symposium for two days. The farms that displayed fish were members of WAFICOS. Fingerlings and table fish were displayed. The fingerlings were given as a door prize and table fish brought for display was sold to participants (see table 2). All the fish served at meal times during the symposium was farmed fish.

Table 1. Papers and presentations at the 2010 Symposium

Presentation	**Name and Institution**
First Session: Opening Remarks	
1. **Welcome Remarks**: A Brief About WAFICOS.	*P. Ssebinyansi*, Chairman WAFICOS
2. Opening Remarks:	*Hon. F. Mukisa*, Minister of State for Agriculture
3. **Key Note Address**: Aquaculture Technological Development - Developments and Challenges.	G. Atukunda, Head Aquaculture, NARO
4. General Discussion about Issues and Challenges Faced by Fish Farmers.	*Hon. F. Mukisa*, Minister of State for Agriculture and *J. J. Otim*, Presidential Advisor on Agriculture
Second Session: Key Production Factors Affecting the Viability of Farming Fish Commercially.	
5. Water Supply and Availability for Fish Farms.	*E.W. Tollner*, University of Georgia, USA/ AquaCRSP
6. Quality of Pond Construction and its Influence on Production and Returns: Recommendations and Farmers Experience.	*Peter Ssebinyansi*, Mpigi Fish Farm.
7. Low Survival Rates in Grow-Out Fish Ponds: Common Causes and Solutions.	Maurice Ssebisubi, Aquaculture Management Consultants, Ltd.
8. Technical Information Sources for Fish Farmers.	Gertrude Atukunda, Aquaculture Research and Development Center, Kajjansi (NAFIRRI-NARO.)
9. Production of High Quality Tilapia Fingerlings	Agnes Atuhaire, Source of Nile Fish Farm
10. Criteria and Best Management Practice Requirements for the Production of High Quality Catfish Fingerlings	Odhiambo Daniel, Kabeihura Fish Farm.
11. Dealing with the Challenges of Transforming from Subsistence to Commercial Fish Farming: Experiences from Eastern Uganda	A. Owori-Wadunde, Aquaculture Research and Development Center, Kajjansi (NAFIRRI-NARO)
12. Production of Nile Tilapia (*Oreochromis niloticus*) in Lake Cages.	*Abudala Napuru*, Source of Nile Fish Farm.
Third Session: Challenges Affecting Fish Farmers Access to Inputs and the Implications on Enterprise Viability and Quality of Service Delivery.	
13. The Challenges Faced in Investing in Commercial Fish Feed Production: A New Industry in Uganda and the Potential Benefits of Floating Fish Feeds for the Developing Aquaculture Sector	Karen. L. Veverica, Ugachick Poultry Breeders Ltd.
14. The Effect of Inadequate Seed Supply on Enterprise Viability: The Cost of 'Come Tomorrow'	Kizito Ssentamu, Wakiso Fish Farm
15. Accessing Finance to Meet Operational Costs	Nafula Owori, 21ACC, Ltd.
16. Mechanisation: Adaptations for the Construction Commercial Fish Ponds	Ssimbwa M., AETREC, Namalere (NARO)
17. The Challenges of Restarting Aquaculture and the Potential for its Commercialization in Previous War Affected Areas of Northern and North East Uganda: Farmers' Perspective	*John Walakira*, for Farmers, Northern Uganda

Fourth Session: Markets, Marketing and Market Information.	
18. Building Markets and Marketing of Farmed Table Fish: Successes and Challenges	Simon Owani, WAFICOS
19. Fish Safety - Quality Aspects	*Phillip Borel*, Greenfields (U) Ltd.
20. Regional Market Study for Farmed fish	Christopher Dhatemwa, UFPEA
21. The Benefits of Forming Cooperatives for Commercial Farmers	Bernard Tayebwa, Uganda Cooperative Alliance
22. Principles of Cooperation in Aquaculture	*Joseph Molnar*, Auburn University/ CRSP
23. WAFICOS Proposed Way Forward	Tom Musoke, WAFICOS
Fifth Session: Public Services to the Aquaculture Sector.	
24. USAID-LEAD Project – Objectives and Approach to Improving Livelihoods through Commercial Aquaculture	*Jacob Olwo*, USAID LEAD
25. WFP's Role in Uganda Aquaculture	*Pius Kwesiga*, World Food Programme
Closing Session.	
Wrap Up Open Discussion	*Professor J. J. Otim,* Presidential Advisor on Agriculture
Closing Remarks	
Door Prize	

Table 2: Exhibitors during the Jan 2010 Symposium**.**

Exhibitor	Products
1. Ugachick Poultry Breeders Limited	Floating fish feeds
2. Crest Tanks Ltd	Water tanks and holding facilities
3. Uganda Oxygen	Oxygen cylinders and refills
4. Pets Alley	Fish aquaria and filter systems
5. Aquaculture Management Consultants	Fish farming guides and advisory products
6. AETREC (NARO) Namalere	Walking tractor for compacting ponds
7. Edhron Enterprises	Catfish fillets, processing and packaging
8. WAFICOS	Live fish sales
9. NAFIRRI (NARO)	Research journals
10. SON Fish Farm	Fish feeds and fingerlings sale
11. Uganda Fish Net Manufacturers	Fish seine nets and cages
12. Ndejje Fish Farm	Live fish

The 2010 Study Tour

One tour was organised that attracted 59 Ugandan participants. The overall objective of the study tour was to expose participants to developments in the sector as well as allow interactive contact between input suppliers, farmers and service providers. The places visited were Tende Innovation Fish farm and Training Center (TIFTC), a catfish hatchery that also operates as a farmer sponsored-and-run farmer field school, Greenfields (U) Limited a fish processing plant that processes farmed fish for local consumption and regional export and Uganda Fish Net Manufacturers Limited that manufactures netting, pond seines and cages.

The 2011 Symposium and Trade Fair

In the year 2010 the key challenges that affected farmers were associated with the use of commercial feeds (including the new floating feed), its use and obtaining good returns. The price of feed had gone up due to a general increase in the cost of ingredients as well as due to the costs of feed manufacture. On the other hand, the price of fish had remained stable. Thus there was an obvious need in improving the efficiency of production as well as obtaining the best price possible of the farmed product and having quick sales.

Thus, the theme of the fourth 2011 symposium was **'Viable Fish Farming'** and the following were the key issues discussed:

1. Production Planning and Management
2. Fish Feeds and Feeding.
3. Value Addition and Marketing of Farmed Fish.
4. Current Support Services to the Aquaculture Private-Sector.

III.

The 2011 Symposium: There were a total of 19 presentations under the above mentioned four specific objectives of this year's symposium.

Table 3. Papers Presented at the 2011 Symposium

ID	Topic	Name of Presenter	Institution
SESSION ONE: OPENING			
1	Welcome Remarks	Paul Ssebinayansi	Chairman, WAFICOS
	Opening: Key Note Address	Mr. Jackson Wadanya	Assistant Commissioner of Fisheries, MAAIF
	General Discussion	Mr. Tom Musoke	Secretary, WAFICOS
SESSION TWO: PRODUCTION PLANNING AND MANAGMENT			
2	Key Issues for Aquaculture Feasibility Analysis and Business Planning in Uganda	Dr. Nelly Isyagi	Aquaculture Management Consultants, Ltd
3	The Potential of Fish Farming in Central Uganda	Dr. Theodora Hyuha	Makerere University
4	Projecting Input and Production Requirements: A Necessity for Successful Commercial Fish farming and Quality Service Delivery	Rita Amolo	Aquaculture Management Consultants, Ltd
5	The Practicalities of Mobilizing Resources to Establish a Commercial Group Owned Fish Farm; Implications on Returns and Viability.	Mr. Nyanzi Abdul	Farmer, Jinja
	General Discussion	Mr. Tom Musoke	Secretary, WAFICOS
SESSION THREE: FISH FEEDS AND FEEDING			
6	Factors Affecting the Performance of Commercial Floating Fish Feeds	Karen L Veverica	Ugachick Poultry Breeders Ltd. / Auburn University
7	Why does Fish Feed Cost so Much?	Karen L Veverica	Ugachick Poultry Breeders Ltd. / Auburn University
8	The Benefits and Risks of Making and Using On-Farm Fish Feeds – A Farmer's Experience	Rhona Nabukeera	Sustainable Commercial Aquaculture for Poverty Alleviation (SCAPA) Project
9	Novus, Making a Difference in Nutrition	David Nyagaka	Novus International – Kenya Office.
10	The Performance of Ugachick Floating Fish Feeds: A Farmers Perspective	Daniel Ojiambo	Kabeihura fish farm, Bushenyi
11	2010 Enterprise Budgets Catfish Grow-Out	Daniel Odhjiambo	Kabeihura fish farm, Bushenyi
12	2010 Enterprise Budgets Tilapia Grow-Out	Biira Yazeri	Kireka Fish Farm
13	2010 Enterprise Budgets Tilapia Nursery	Kiddu Ben	WAFICOS
	General Discussion	Mr. Tom Musoke	Secretary, WAFICOS
SESSION FOUR: VALUE ADDITION AND MARKETING OF FARMED FISH			
14	Creative Marketing	Tom Musoke	Kabaganda Fish Farm
15	Value-Addition, Preservation and Marketing	Dr. Margaret Maseette	FBRC-Kawanda

ID	Topic	Name of Presenter	Institution
	Discussion: Times when I Sold at a Profit and at a Loss.	Mrs. Mary Zaramba	Fish Farmer, WAFICOS
SESSION FIVE: SERVICES TO THE SECTOR			
16	Financing Aquaculture	Ms. Sabano Mwaka Ann Marie.	Agricultural Credit Officer, Centenary Bank.
17	Helping Build Aquaculture Enterprises, Success and Challenges	Jacob Olwoo	USAID LEAD
18	Deep Blue Aquatic Systems	Brynn Simpson	Deep Blue Aquatic Systems, South Africa
19	Certification for Aquaculture Professionals	Karen Veverica,	Auburn University, USA.
20	EU Study on Promoting Commercial Aquaculture in Uganda	Malcolm Dickson.	EU Mission/COWI
	General Discussion	Mr. Tom Musoke	Secretary, WAFICOS
SESSION SIX: CLOSING			
	Question Answer Time and Wrap-up Discussions	Mr. Tom Musoke	Secretary, WAFICOS
	Prize Draws	Mr. Paul Ssebinaynsi and Mr. David Tilia	Chairman WAFICOS and Principal Fisheries Officer, MAAIF
	Closing Remarks	Mr. David Tilia	Principal Fisheries Officer, MAAIF

Among the participants were two Kenyans and the EU Mission undertaking a study on 'Promoting Commercial Aquaculture in Uganda'. The study was specifically scheduled so that the mission could attend the symposium and obtain up-date information on Ugandan aquaculture from farmers and other key stakeholders.

The 2011 Trade Fair

There were 11 exhibitors at the trade (see table 4). There was a strong emphasis on the marketing of farmed fish this year. The table fish and fish-products brought to the fair were all sold to participants and people from around the UMA show grounds who came to have a look at what was on display. All the fish and fish products served at this year's symposium (both tilapia and catfish) were from WAFICOS farmed fish. One of the exhibitors, Deep Blue Aquatic Systems came in from South Africa.

Table 4: List of Exhibitors

Exhibitor	Products
1. Ugachick Poultry Breeders Limited	Fish feeds and feeding guides.
2. Green Fields Uganda Limited	Fish fingers and fish burgers
3. SON- Source of the Nile fish farm	Whole fresh tilapia on ice.
4. WAFICOS	Live table size catfish and tilapia. Smoked whole catfish.
5. Aquaculture Management Consultants	Aquaculture equipment and aquaria
6. Deep Blue Aquatic Systems (in collaboration with Aquaculture Management Consultants)	Aquaculture and live fish holding systems
7. Sun Fish Farm	Live fish haulage truck
8. Nile Crocodile Park.	Books. The Best Options for Africa
9. National Fisheries Resources Institute	Research papers/journals
10. Tende Innovation Fish Farm and Training Center	Live catfish fingerlings.
11. Uganda Fish Net Manufacturers	Fish seine nets, predator nets and cages

The 2011 Study Tour

The farmers study tour in 2011 was expanded. In the previous year (2010) participants of the tour noted that there was a lot to learn from the sites visited and it was not worth it for them to visit several sites like 'tourists' in a day. Thus the objectives of the 2011 were not just to show-case but demonstrate best practices that participants could pick up for adoption. The demonstrations were undertaken by farmers themselves with the assistance of technical personnel from WAFICOS and Aquaculture Management Consultants limited. Training hand-outs were prepared for distribution by Aquaculture Management Consultants Limited.

The sites visited were:

1. *Route 1: Kabaganda Fish Farm and Ugachick Poultry Breeders Limited* that demonstrated tilapia nursery management, commercial fish feed production and the processing of catfish fillets.
2. *Route 2: ARDC – Aquaculture Research and Development Center (ARDC) Kajjansi and Pearl Fishing and Aquaculture Limited*. This tour showcased the new research facilities at the Aquaculture Research and Development Center and demonstrated feed-based tilapia and catfish pond grow-out management.
3. *Route 3: Living Waters Fish Farm and Kireka Fish Farm*. This tour demonstrated feed-based tilapia and catfish pond grow-out management, siting and setting-out of new ponds, use of the commercial pond seine and catfish hatchery management.

As a sequel attendance of the Kenyan farmers to the 2011 symposium was a study tour organised for a group of Kenyan fish farmers and extension agents. Table 5 gives an overview of the places visited.

Table 5. Study Tour for Kenyan Farmers

Day	Venue	Type of Farm
One	• Arrival in Kampala	
Two	• Source of Nile Farm, Buikwe	• Tilapia hatchery and cage culture.
	• Living Waters, Mukon	• Catfish and Tilapia grow-out.
	• Kireka Fish Farm, Wakiso	• Smallholder catfish hatchery.
Three	• Kabaganda Fish Farm, Wakiso	• Tilapia Nursery
	• Ugachick Poultry Breeders Limited, Wakiso	• Fish Feed Factory.
	• Travel to Bushenyi	
Four	• Kabeihura Fish Farm and fish farmer out-growers , Bushenyi	• Catfish hatchery • Tilapia and Catfish grow-out
Five	• Travel Back to Kampala	• Tilapia Nursery
	• Mpigi Fish Farm, Mpigi	
Six	• Return to Nairobi	

Other Seminars and Tours

The outreach component participated in coordinating, offering technical support, preparation of training material and compiling documentation for the seminars and field visits:

1. Review of the questionnaire for the marketing study.
2. Seminar by Tollner at MUK and Kabanyolo.
3. Field visits of Hydrology MUK to potential study sites in Gulu, Wakiso, MItyana, Mukono and Buikwe.
4. Sourcing of cages, fingerlings, stocking and sampling of CRSP trial cages.

IV.

CONCLUSION

Participants considered the symposia and study tours a general success. The key factors that were used to define success were the relevance of the presentations and demonstrations to the prevailing challenges that farmers and other stakeholder in the sector were facing. The effectiveness of farmer-to-farmer dissemination was fully realised as most of the presentation were by farmers, the discussions during the plenary session were led by the farmers themselves and the key facilitators on farms during the study tours, were the farmers themselves. The technical personnel only came in to supplement and explain the principals upon which the technologies were based and issues that affected their application on-farms.

The role and capability of the private sector where appropriately supported in dissemination of technical information and training is also illustrated. WAFICOS, as producers and aquaculture service providers are the aquaculture private-sector. Participants paid attendance to the symposia and trade fair as did exhibitors. Likewise is the level of support the program has received from Aquaculture Management Consultants Limited. This shows that where quality services are provided that are result oriented, farmers and other service providers in the private sector can support these initiatives to make them sustainable.

The outreach program has been able to disseminate information to stakeholders directly. Proceedings have been disseminated via CD to all attendees of the symposia as well as personnel in key government offices notably the Ministry of Agriculture, Ministry of Finance Economic Planning and Development, the National Agriculture Research Organisation 9head office and its centres involved in aquaculture), Makerere University, Fisheries Training Institute, District Fisheries Officers, FAO, USAID LEAD, Banks, etc. Proceedings of the symposia have also been disseminated via the internet to the wider African and international community via the SARNISSA website that is a widely consulted aquaculture interactive site in Africa.

The outreach strategy adopted can be considered a success. Dissemination can be undertaken through direct contact training seminars between CRSP personnel and farmers, farmer-to-farmer and via media-based PC tools.

The project anticipated that major benefits of the project would be:

1. Information and improved production for individual farmers and rural communities.
2. Increase in the membership of fish farmer groups as a result of the symposia.
3. Service providers and suppliers of inputs gaining recognition and possibly clients.

The benefits that actually accrued based on the response of WAFICOS members (Quote):

1. It has become a forum where farmers and others involved in the industry discuss up-to-date issues affecting them. The factors that influence performance are dynamic. If one is to stay in business, then problems have to be solved along the way and opportunities identified early and are taken advantage of. The papers presented and study tours focus at key issues that arose the previous year. Hence, farmers and service providers mention this as among the key benefits of the symposium
2. WAFICOS membership has increased. So has the credibility of the association. Agencies notably NGO's and NAADS (National agricultural advisory services) now cross-check with WAFICOS when vetting tenders (hatcheries) to supply fingerlings asking the association to verify whether the farm exists and what sort of fingerling quality the farms tend to produce.
3. WAFICOS now asked officially by training institutions (Fisheries Training Institute and Makerere University) for placement of interns.
4. The farms and other establishments visited during the study tours have registered increased sales. Such farms also have more visitors and training institutions seek to attach interns or conduct study tours on these establishments.
5. More farmers demanding for services from WAFICOS which include information, technical services and inputs. Among the key success is WAFICOS office becoming an official outlet for Ugachick feed whereby farmers can buy the feed at wholesale price. This has enabled farmers identify loopholes and opportunities beyond the farm level. Several farmers have taken advantage of this and have established businesses (i.e. marketing, inputs) and are negotiating getting better prices for their fish.
6. WAFICOS ties with other institutions, (Government, donor, NGO's, training institutions) have been strengthened. The relationship is increasingly becoming two way as they realize that WAFICOS has useful information to give that they would otherwise have no access to, thus has a contribution to the sector. The relationship less of one where they look as the association as a 'beggar' that can only survive on their handouts.
7. WAFICOS and other farmers have learnt more about what other farmers are doing from the symposium.
8. Farmers have learnt new technologies and their application through the symposia and study tours. Adoption rates of appropriate technologies has consequently increased (e.g. use of feed, pond construction techniques) as has been observed when WAFICOS technical staff visit farm or when farmers come to the WAFICOS office to register and seek services. Farmers also now appreciate why certain things are the way they are because during the symposia different stakeholders' discuss the issues affecting them not just services offered. The discussions get to the heart of the matter. Hence, there are more farmers who visit the WAFICOS who now realize the onus is on them to make their farms viable by adopting best techniques, improving efficiency of production rather than waiting for government/donor grants, asking for loans or complaining about feed prices being too high. They understand why things are the way they e.g. costs of ingredients, what is involved in getting loans and

that one should only think once the business proves itself profitably and running. In essence, those who have attended the symposia are becoming aquaculture business men and women.

9. WAFICOS is now known country-wide and membership has increased beyond the original stipulated geographical region of Mpigi, Wakiso, and Kampala. Membership is now country-wide and services delivered to members where-ever they are. Farmers pay for transport and are willing to do so because of the reliability and quality of services offered.

ACKNOWLEDGMENTS

Additional Funding was provided by USAID Livelihood and Enterprises for Agricultural Development (LEAD), Food and Agriculture Organisation (FAO), and World Food Program (WFP).

Additional logistical support provided by: Aquaculture Management Consultants Limited, the National Agricultural Research Organisation (NARO) and Uganda Veterinary Association.

Incorporation of Tilapia (*Oreochromis Niloticus*) and Sahar (*Tor Putitora*) into the Existing Carp Polyculture System of Nepal

Production System Design and Best Management Alternatives/Experiment/09BMA03UM

Madhav K. Shrestha and Ramesh Jaiswal
Institute of Agriculture and Animal Sciences
Rampur, Chitwan, Nepal

Liu Liping
Shanghai Ocean University
Shanghai, China

James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

ABSTRACT

An experiment was conducted for 180 days in 12 earthen ponds, 110-150 m^2 in surface area and 1.0 m in depth, at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal to assess the growth, production, and productivity of carps, tilapia (*Oreochromis niloticus*), and sahar (*Tor putitora)* in different polyculture combinations. There were one control and three treatments with three replicates each: (1) Existing carp polyculture (silver carp, bighead carp, common carp, grass carp, rohu, and mrigal in the ratio of 3:2:2.5:0.5:1:1 and stocking density of 7000 fish ha^{-1}) (control); (2) Control + tilapia (3000 ha^{-1}); (3) Control + tilapia (3000 ha^{-1}) + sahar (500 ha^{-1}); (4) Control + tilapia (3000 ha^{-1}) + sahar (1000 ha^{-1}). The ponds were fertilized weekly at 4 kg N and 1 kg P ha^{-1} d^{-1} using diammonium phosphate (DAP) and urea. Fish were fed on alternate days at 0900–1000 h, with a locally made pelleted feed (20% crude protein) at rates of 2% body weight per day. Feed rations were adjusted monthly based on sampling weights.

Survival of carps (68-89%), tilapia (69-83%) and sahar (43-49%) were not significantly different among treatments. All carp species showed better performance in all treatments than sahar or tilapia with a daily weight gain of 1.0 to 2.4 g d^{-1}. The combined net and gross yields of all carps in T_1 and T_2 were significantly higher than T_3 and T_4 ($p<0.05$). The combined net and gross yields were significantly higher in T_2 than other treatments ($p<0.05$), which were not significantly different from each other ($p>0.05$). The overall FCR was significantly lower in T_2 (1.0±0.0) than T_4 (1.4±0.1) ($p<0.05$), whereas there were no significant differences among other treatments. The number of tilapia recruits was significantly higher in T_2 (798±32) than T_3 (676±51) and T_4 (603±72). There were no significant differences in any water quality parameters among treatments. Pond water temperature remained above 20°C throughout the experimental period. Dissolved oxygen concentrations in the T_3 and T_4 ponds were consistently low during most of the experimental period and caused mortality of sahar and silver carps. All treatments produced a positive gross margin, with the significantly highest gross margin in T_2 (NRs 793,800 ha^{-1} $year^{-1}$). Additions of Nile tilapia in the semi-intensive carp polyculture ponds can significantly increase the fish production up to 57% and net returns up to 61%. However, high stocking densities caused by adding sahar to the polyculture reduced economic performance.

INTRODUCTION

Inland aquaculture and fisheries are the only source of fish production in Nepal. Total fish production is 48,230 mt, with 21,500 mt (about 45%) coming from capture fisheries (DoFD, 2010). Current annual fish production of Nepal aquaculture systems is about 3.3 t/ha (DoFD, 2010). Increasing fish productivity as well as total production in country is a challenging task and necessary in order to provide for increasing demand for fish as food without increasing import from neighboring countries. Nile tilapia (*Oreochromis niloticus*) was introduced in Nepal in 1985 (Pantha, 1993), however, it remained in government control for more than 10 years (Shrestha and Bhujel, 1999). We have worked on tilapia and sahar (*Tor putitora*) combinations in polyculture to control excessive recruitment of tilapia and also to provide additional species to increase productivity and to promote culture of high value fish that are indigenous. Sahar can control tilapia fry in mixed sex culture (Paudel et al., 2007; Rai et al., 2007; Yadav et al., 2007). Polyculture of sahar with mixed-sex tilapia increased fish yield significantly compared to tilapia monoculture (Shrestha et al., 2011). Growth of sahar is higher in tropical and subtropical ponds than in cages in lakes near Pokhara and also in suspended cages in ponds (Shrestha et al., 2005; 2007; Bista et al., 2001; 2007).

Semi-intensive carp polyculture is an established and recommended system in tropical and subtropical region of Nepal using fertilized ponds with partial feed supplementation. The species are: common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), rohu (*Labeo rohita*), naini/mrigal (*Cirrhinus mrigala*), and Bhakur/Catla (*Catla catla*). Though all seven species are recommended in certain ratios with a combined density of 7000 fish/ha (Pandey et al., 2007), fingerlings of all species are rarely available when needed for stocking. In most of the cases, the number of species cultured ranges from four to six. Addition of well proven species (such as tilapia and sahar) with increased stocking density into the existing carp production system can have a positive impact by increased productivity and economic value.

The purpose of this study was to incorporate tilapia and sahar into carp polyculture to improve production in order to better develop the model for best production and to determine the costs and benefits of various polyculture combinations.

The objectives of this study were:

I. To assess the growth, production and productivity of carps, tilapia and sahar in different polyculture combinations;
II. To assess nutrient recovery in each system
III. To determine cost and benefits of fish production in each polyculture system;
IV. To assess and compare water quality produced by each polyculture treatment;
V. To promote those results in a workshop targeted on polyculture and family nutrition.

METHODS AND MATERIALS

This experiment was conducted in 12 earthen ponds, 110-150 m^2 in surface area and 1.0 m in depth, at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal. The experiment was conducted in completely randomized design using one control and three treatments with three replicate each: (1) Existing carp polyculture (7000 fish ha^{-1}) (control); (2) Control + tilapia (3000 ha^{-1}); (3) Control + tilapia (3000 ha^{-1}) + sahar (500 ha^{-1}); (4) Control + tilapia (3000 ha^{-1}) + sahar (1000/ha^{-1}). Ponds were drained and filled with canal water two weeks before fish stocking. They were fertilized at 4 kg N and 1 kg P ha^{-1} d^{-1} for 7 days with diammonium phosphate (DAP) and urea. A feeding tray was placed in each pond to confine feeding. A wooden platform was constructed in the ponds for feeding and water sampling. Water depth was maintained 1.0 m in all ponds by biweekly topping with canal water to replace water loss due to evaporation and seepage. Fish were fed once on alternate days at 0900–1000 h, with a locally made pelleted feed (20% crude protein) at rates of 2% body weight per day. Feed rations were

adjusted monthly based on sampling weights. Ponds were fertilized weekly using DAP and urea at rates of 4 kg N and 1 kg P ha^{-1} d^{-1}.

Fingerlings of silver carp, bighead carp, common carp, grass carp, rohu, and mrigal (average weights 24.1±2.2 g, 36.7±4.6 g, 20.4±2.9 g, 39.7±3.8 g, 9.7±1.4 g and 6.9±0.7 g, respectively) were stocked in all ponds. The stocking ratio was 3:2:2.5:0.5:1:1 in all ponds. Similarly, Nile tilapia and sahar (average weights 11.6±1.6 g and 3.4±0.4 g, respectively) were added in treatment ponds. Fish were stocked on 25 February 2011 and harvested on 26 August 2011.

Weekly and biweekly measurements of water quality parameters were conducted at 0600–0800 h starting from 25 February 2011. Water temperature, dissolved oxygen (DO), pH, and Secchi disk depth were measured in situ weekly using a Thermometer (Lutron TM-936 model), DO meter (Lutron DO-5509 model), pH meter (Lutron YK-21 PH model), and Secchi disk, respectively. Water samples were collected biweekly from each pond using a column sampler and analyzed for total alkalinity, total ammonium nitrogen (TAN), nitrite nitrogen (NO_2-N), soluble reactive phosphorous (SRP), and chlorophyll a using standard methods (APHA, 1985).

A simple economic analysis was conducted based on farm-gate prices for harvested fish and market prices for all costs in Nepal (Shang, 1990). Farm gate prices of sahar, tilapia, carp were 300, 200 and 150-200 NRs kg^{-1} ($1 US = 73 NRs), respectively. Prices for sahar, tilapia, and carps fingerlings were 5, 2 and 2 NRs $piece^{-1}$, respectively. Prices for feed, DAP, and urea were 20, 45, and 30 NRs kg^{-1}, respectively. The calculation for cost of working capital was based on an annual interest rate of 10%.

Data were analyzed statistically by one way analysis of variance (ANOVA), using SPSS (version 16.0) statistical software (SPSS Inc., Chicago). Arcsine transformations were performed on percent data. Differences were considered significant at the 95% confidence level ($P < 0.05$). All means were given with ±1 standard error (S.E.).

RESULTS

The survival rate of stocked Nile tilapia ranged from 69.4 to 83.2%, without significant differences among treatments (Table 1). There were no significant differences in mean harvest weight, daily weight gain, net fish yield (NFY) and gross fish yield (GFY) of tilapia among treatments. The survival of sahar was very low, ranging from 42.9 to 48.5%, without significant differences among treatments (Table 1). The daily weight gain of sahar ranged from 0.3 to 0.4 g d^{-1} (Figure 1), also without significant differences among treatments. The GFY and NFY of sahar ranged from 0.03 to 0.06 and 0.02 to 0.03 t ha^{-1} yr^{-1}, respectively and was significantly different ($p<0.05$) between treatments with high yield at the highest sahar density. The growth trend of Nile tilapia was about 1 g/d (Figure 2).

The survival of individual carps was not significantly different among treatments (Table 2). However, the overall survival of all carps was significantly higher in T_2 than T_4, whereas there were no significant differences among other treatments. All carp species showed better performance than tilapia or sahar in all treatments with a daily weight gain of 1.0 to 2.4 g. The daily weight gain for silver carp ranged from 1.8 to 2.3 g , with a significantly higher mean daily weight gain in T_4 than other treatments ($p<0.05$). Similarly, the daily weight gain of other carps ranged from 1.0 to 2.0 g, with no significant differences between treatments (Table 2). There were no significant differences in GFY or NFY of silver carp, bighead carp, rohu and mrigal among all treatments. The combined net and gross yields of all carps in T_1 and T_2 were significantly higher than T_3 and T_4 ($p<0.05$).

The combined net and gross yields of all species in a treatment were significantly higher in T_2 than other treatments ($p<0.05$, Table 3). The overall food conversion rate (FCR) was significantly lower in T_2 than T_4 ($p<0.05$), whereas there were no significant differences among other treatments (Table 4). The number of tilapia recruits was significantly higher in T_2 (798±32) than T_3 (676±51) and T_4 (603±72) (Table 3). There were no significant differences in the mean weight and NFY of recruits among treatments.

Weekly and fortnightly means of water quality parameters are presented in Table 5 and Figures 3 to 9. Analyses showed that there were no significant differences in any water quality parameters among treatments ($p>0.05$; Table 5). Most water quality parameters tended to fluctuate throughout the experimental period depending upon weather and nutrient supply. Pond water temperature remained above 20°C and increased gradually from 20.7 to 31.9°C throughout the experiment (Figure 3). Dissolved oxygen concentrations in the T_3 and T_4 ponds were consistently low during most of the experiment. Pond water pH ranged from 7.4 to 7.6, total alkalinity from 190.6 to 202.0 mg L^{-1} as $CaCO_3$ and chlorophyll-a from 155.8 to 198.3 mg m^{-3}. Total ammonium nitrogen increased from the beginning to the middle part of the experiment, and then decreased dramatically to the end of the experiment (Figure 7).

Results of economic analysis showed that all treatments produced a positive gross margin (Table 6), with the highest gross margin in T_2 (793800 NRs ha^{-1} $year^{-1}$), intermediate in T_3 (614100 NRs ha^{-1} $year^{-1}$) and T_4 (651500 NRs ha^{-1} $year^{-1}$), and lowest in T_1 (491600 NRs ha^{-1} $year^{-1}$) ($p<0.05$).

Our proposal for this experiment had 5 objectives, and not all were accomplished in the study reported here. Unfortunately, we had difficulties completing the experiment because of flooding at the facility and theft. Therefore, although we have some reasonable results reported here, we have just completed the on-station experiment again, and now we are doing the on farm work. Objective 2, which is a Masters thesis project to assess nutrient recovery, is part of the on-station study which we just completed and will also be part of the on-farm study. The MS student is currently analyzing the pond bottom soil, feed, and fish samples.

A one-day workshop held on January 11 was jointly organized by IAAS and the Nepal Fisheries Society to promote the results of this experiment. The workshop was attended by 47 participants (8 women) including researchers, government and extension officers, farmers, and businessmen. Technical presentations covered recent work on the CRSP tilapia-sahar- carp polyculture system, cage-pond systems for small farmers, and a comparative analysis of production and economics for cage-pond farmers versus pond farmers. An open discussion on how to use these outcomes followed the technical presentations. Government, extension, and research agency representatives showed interest to take up these outcomes.

DISCUSSION

The purpose of this study was to increase the production of existing semi-intensive carp polyculture of Nepal by addition of new species, tilapia, and sahar. The daily weight gain of Nile tilapia was 0.8-1.0 g d^{-1}, which was higher than a grass carp-tilapia polyculture system (0.2-0.5 g) (Pandit et al., 2004), and similar with tilapia-sahar polyculture systems (1.15 g; Acharya et al., 2007, and 0.6-0.9 g; Shrestha et al., 2011). Similarly, the daily weight gain of sahar was 0.3 to 0.4 g, which was similar to tilapia-sahar polyculture system (0.3-0.4 g d^{-1}) (Shrestha et al., 2011) and lower than monoculture (0.55-0.77 g d^{-1}) (Islam, 2002). The survival of sahar was very low, ranging from 43-49%. This survival was lower than that reported by Acharya et al. (2007), but similar to Shrestha et al. (2011) (39-56%) in tilapia-sahar polyculture systems. The lower survival of sahar was due to mass mortality of sahar by low dissolved oxygen. DO in pond water was consistently lower in ponds of T_3 and T_4, and it decreased to a minimum of 0.1 mg L^{-1} on some dates.

Gross carp yields (3.4 to 4.1 t ha^{-1} yr^{-1}) were higher than the average productivity in carp polyculture ponds in Nepal (3.0 t/ha/yr) (DoFD, 2010). Net yields of all carps were significantly higher in carp-tilapia

polyculture than in other treatments. Growth performances of carp species were not affected by addition of tilapia, and there was no competition between carp and tilapia for pond resources. We can add Nile tilapia to carp polyculture ponds up to a certain density to enhance overall production.

Most water quality parameters in all ponds were within acceptable ranges for fish culture. Water quality was not drastically affected by stocking densities of fishes. However, frequent DO depletion was observed in the high stocking density treatments, T_3 and T_4. The FCRs recorded were fairly low, probably due to alternate day feeding.

Income in these experiments was estimated by simple budget analysis. Fixed costs were not included in the analysis as we intended to only compare relative differences in efficiency between the treatments, and we assumed fixed costs to be similar for all the treatments. Cost estimation was based on local market prices of fingerlings, fertilizers, lime, and labor wages. Results showed that all treatments produced positive net returns ranging from 491,600 to 793,800 NRs. However, the carp-tilapia combination (T_2) produced significantly higher net return than other treatments.

This study clearly demonstrated that addition of Nile tilapia in the semi-intensive carp polyculture ponds increased production up to 57% and net returns up to 61% without affecting water quality. However, the addition of both tilapia and sahar in semi-intensive carp polyculture ponds caused some water quality problems, especially depleting DO in the pond. Thus, it is necessary to fine-tune the ratio of carps, tilapia, and sahar in polyculture if all of these species are stocked. This new polyculture system provides an alternative species for farmers and allows for diversification of species in carp polyculture ponds. Finally, further research on the improved survival rate and growth of sahar in carp polyculture ponds is needed.

ANTICIPATED BENEFITS

The results of this study will provide an additional species in polyculture systems of Nepal with increased productivity, production, and income. These changes will add high valued fish into the culture system and will supplement income. As carp polyculture is an established system, increasing species will be easy for fish farmers to adopt. If sahar and tilapia are cultured, it will also help in production of sahar and decrease fishing pressure in nature. These changes will benefit fish culturists in south Asia and other countries where carp culture is popular. Knowledge on polyculture and expansion to endemic species not only benefits Nepal, but sustainable aquaculture systems throughout the developed world as well. Immediate impact will be measured by increased production and economic returns in on-farm trials for the different polyculture systems.

ACKNOWLEDGMENTS

The authors wish to acknowledge the support from the Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal, and the Shanghai Ocean University, Shanghai, China. Mr. Narayan P. Pandit of IAAS is thanked for his contributions to data analysis and report preparation.

LITERATURE CITED

Acharya, D., D.K. Jha, M.K. Shrestha, and N.R. Devkota, 2007. Polyculture of sahar (*Tor putitora*) and mixed-sex Nile tilapia (*Oreochromis niloticus*) in Chitwan, Nepal. In: R.B. Thapa, and M.D. Sharma (Editors), IAAS Research Advances, 1, pp. 187–193.

APHA, 1985. Standard Methods for the Examination of Water and Waste Water (16th edition). American Public Health Association, Washington D.C. 1268 pp.

Bista J.D., S.K. Wagle, M.K. Shrestha, and A.B. Thapa, 2007. Evaluation of growth performance of Himalayan sahar (*Tor putitora*) for aquaculture development in mid hills and southern plain of Nepal. Seventh National Workshop on Livestock and Fisheries Research, June 25-27, 2007. Abstracts, p. 12.

Bista, J., B.R. Pradhan, A.K. Rai, R.K. Shrestha, and T.B. Gurung, 2001. Nutrition, feed, feeding of golden mahaseer (*Tor putitora*) for domestication and production in Nepal. Symposium on coldwater fishes in the trans Himalayan region, 10-14 July, Kathmandu, Nepal.

DoFD (Department of Fisheries Development), 2010. Annual report. Directorate of Fisheries Development, Balaju. Department of Agriculture, Ministry of Agriculture and Co-operatives, Government of Nepal.

Islam, M.S., 2002. Evaluation of supplementary feeds for semi-intensive pond culture of mahseer, *Tor putitora* (Hamilton). Aquaculture, 212:263–276.

Pandey, C.M., M.K. Shrestha, C.N.R. Yadav, and S. Rai, 2007. Productivity of carp polyculture system under farmers' pond condition in sub tropical Nepal. In: R.B. Thapa and M.D. Sharma (Editors), IAAS Research Advances Vol. 1. IAAS, Rampur, Chitwan, Nepal, pp. 175-179.

Pandit, N.P., M.K. Shrestha, Y. Yi, and J.S. Diana, 2004, Polyculture of grass carp and Nile tilapia with napier grass as the sole nutrient input in the subtropical climate of Nepal. In: R. Bolivar, G. Mair, and K. Fitzsimmons (Editors), New Dimension in Farmed tilapia, Proceedings of 6th International Symposium on Tilapia in Aquaculture (ISTA 6), pp. 558-573.

Pantha, M.B., 1993. Aquafeeds and feeding strategy in Nepal. In: M. W. New, A.G.T. Tacon and I. Csavas (eds.) Farm Made Aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consolation on Farm-Made Aquafeeds, 14-18 December 1992, Bangkok, Thailand. FAO-RAPA/AADCP, Bangkok. pp. 24-60.

Paudel, J.K., D.K. Jha, M.K. Shrestha, and J.D. Bista, 2007. Growth performance of sahar (*Tor putitora*) in different culture systems in Chitwan, Nepal. In: R.B. Thapa and M.D. Sharma (Editors), IAAS Research Advances Vol 2. IAAS, Rampur, Chitwan, Nepal. pp. 195-200.

Rai S., M.K. Shrestha, D.K. Jha, and D. Acharya, 2007. Growth performance of sahar and mixed-sex Nile tilapia in monoculture and co-culture system in Chitwan. In: R.B. Thapa and M.D. Sharma (Editors), IAAS Research Advances Vol 1. IAAS, Rampur, Chitwan, Nepal. pp. 187-193.

Shang, Y.C., 1990. Aquaculture Economic Analysis: An Introduction. World Aquaculture Society, Baton Rouge, Louisiana, 211 pp.

Shrestha, M.K., and R.C. Bhujel, 1999. A preliminary study on Nile tilapia (*Oreochromis niloticus*) polyculture with common carp (*Cyprinus carpio*) fed with duckweed (*Spirodela*) in Nepal. Asian Fisheries Science, 12: 83-89.

Shrestha, M.K., J.D. Bista**,** and Y. Yi., 2007. Performance of *Tor putitora* in sub tropical climate of Nepal. 8th Asian Fisheries Forum. Asian Fisheries Society and Asian Fisheries Society, Indian Branch, 20-23 November 2007, Kochi, India. Fisheries and Aquaculture: Strategic Outlook for Asia, Book of Abstracts, pp. 250-251.

Shrestha, M.K., N.P. Pandit, Y. Yi, J.S. Diana, and C.K. Lin, 2005. Integrated cage-cum-pond culture system with high-valued sahar (*Tor putitora*) suspended in carp polyculture ponds. In: J. Burright, C. Flemming, and H. Egna (Editors), Twenty-Second Annual Technical Report. Aquaculture CRSP, Oregon State University, Corvallis, Oregon. pp. 97-114.

Shrestha, M.K., R.L. Sharma, K. Gharti and J.S. Diana, 2011. Polyculture of sahar (*Tor putitora*) with mixed-sex Nile tilapia. Aquaculture, 319: 284-289.

Yadav, R.K., M.K. Shrestha, and N.P. Pandit, 2007. Introduction of sahar (*Tor putitora*) in cage-cum-pond integrated system of mixed-sex Nile tilapia (*Oreochromis niloticus*). Our Nature, 5:52-59.

Table 1. Performance of Nile tilapia and sahar in different treatments. Data based on 100 m^2 pond size. Mean values with same superscript in the same row are not significantly different ($p<0.05$).

Parameters	Treatment			
	T_1	T_2	T_3	T_4
Nile Tilapia Stocking				
Total weight (kg)	-	0.2±0.0[a]	0.4±0.1[a]	0.5±0.2[a]
Mean weight (g)	-	8.0±1.8[a]	13.3±3.3[a]	13.6±2.6[a]
Harvest				
Total weight (kg)	-	4.1±0.3[a]	3.2±0.5[a]	4.2±0.3[a]
Mean weight (g)	-	167.7±16.2[a]	150.0±9.1[a]	200.0±31.0[a]
Daily weight gain (g d^{-1})	-	0.9±0.1[a]	0.8±0.1[a]	1.0±0.2[a]
Survival (%)	-	83.2±12.9[a]	69.4±9.9[a]	71.5±11.2[a]
GFY (t ha^{-1} yr^{-1})	-	0.8±0.1[a]	0.6±0.1[a]	0.8±0.1[a]
NFY (t ha^{-1} yr^{-1})	-	0.8±0.1[a]	0.5±0.1[a]	0.7±0.1[a]
Sahar Stocking				
Total weight (kg)	-	-	0.02±0.00[a]	0.02±0.00[a]
Mean weight (g)	-	-	4.2±0.6[a]	2.7±0.1[a]
Harvest				
Total weight (kg)	-	-	0.2±0.0[b]	0.3±0.0[a]
Mean weight (g)	-	-	67.6±1.3[a]	62.1±3.0[a]
Daily weight gain (g d^{-1})	-	-	0.4±0.0[a]	0.3±0.0[a]
Survival (%)	-	-	42.9±7.1[a]	48.5±3.6[a]
GFY (t ha^{-1} yr^{-1})	-	-	0.03±0.00[b]	0.06±0.00[a]
NFY (t ha^{-1} yr^{-1})	-	-	0.02±0.00[b]	0.03±0.00[a]

Table 2. Performance of carps in different treatments. Data based on 100 m^2 pond size. Mean values with same superscript in the same row are not significantly different ($p<0.05$).

Parameters	Treatment			
	T_1	T_2	T_3	T_4
Silver Carp Stocking				
Total weight (kg)	0.5±0.1 [a]	0.5±0.1 [a]	0.5±0.0 [a]	0.7±0.1 [a]
Mean weight (g)	23.1±4.7 [a]	20.7±4.3 [a]	22.1±0.9 [a]	30.4±5.9 [a]
Harvest				
Total weight (kg)	5.0±0.9 [a]	6.0±0.4 [a]	5.5±0.3 [a]	5.0±0.6 [a]
Mean weight (g)	371.8±26.7 [b]	349.0±15.3 [b]	362.2±16.5 [b]	450.5±12.9 [a]
Daily weight gain (g d^{-1})	1.9±0.2 [b]	1.8±0.1 [b]	1.9±0.1 [b]	2.3±0.1 [a]
Survival (%)	61.9±14.2 [a]	77.3±6.6 [a]	66.9±5.4 [a]	50.8±7.0 [a]
GFY (t ha^{-1} yr^{-1})	1.0±0.2 [a]	1.2±0.1 [a]	1.1±0.1 [a]	1.0±0.1 [a]
NFY (t ha^{-1} yr^{-1})	0.9±0.2 [a]	1.1±0.1 [a]	1.0±0.1 [a]	0.9±0.1 [a]
Bighead Carp Stocking				
Total weight (kg)	0.5±0.2 [a]	0.5±0.1 [a]	0.4±0.1 [a]	0.7±0.1 [a]
Mean weight (g)	34.5±10.7 [a]	33.0±9.3 [a]	30.4±6.7 [a]	48.9±10.5 [a]
Harvest				
Total weight (kg)	3.8±0.8 [a]	2.7±0.1 [a]	3.4±0.2 [a]	3.8±0.3 [a]
Mean weight (g)	300.0±70.4 [a]	206.5±3.2 [a]	268.0±24.0 [a]	268.8±57.8 [a]
Daily weight gain (g d^{-1})	1.5±0.4 [a]	1.0±0.0 [a]	1.3±0.1 [a]	1.8±0.4 [a]
Survival (%)	93.1±7.4 [a]	94.2±3.2 [a]	94.8±2.9 [a]	77.2±11.4 [a]
GFY (t ha^{-1} yr^{-1})	0.8±0.2 [a]	0.5±0.0 [a]	0.7±0.0 [a]	0.8±0.1 [a]
NFY (t ha^{-1} yr^{-1})	0.7±0.2 [a]	0.4±0.0 [a]	0.6±0.0 [a]	0.6±0.1 [a]
Common Carp Stocking				
Total weight (kg)	0.3±0.1 [a]	0.4±0.0 [a]	0.3±0.1 [a]	0.5±0.2 [a]
Mean weight (g)	19.8±5.3 [a]	20.2±1.7 [a]	16.0±6.4 [a]	25.7±9.4 [a]
Harvest				
Total weight (kg)	5.7±0.4 [a]	5.2±0.2 [a]	3.9±0.7 [a]	3.9±0.2 [a]
Mean weight (g)	457.4±83.3 [a]	309.3±2.4 [ab]	316.9±22.5 [b]	368.3±7.7 [b]
Daily weight gain (g d^{-1})	2.4±0.5 [a]	1.6±0.0 [a]	1.7±0.1 [a]	1.9±0.1 [a]
Survival (%)	73.5±9.9 [a]	94.6±3.5 [a]	73.1±18.3 [a]	59.1±3.7 [a]
GFY (t ha^{-1} yr^{-1})	1.1±0.1 [a]	1.0±0.0 [ab]	0.8±0.1 [b]	0.8±0.0 [b]
NFY (t ha^{-1} yr^{-1})	1.1±0.1 [a]	1.0±0.0 [ab]	0.7±0.1 [b]	0.7±0.0 [b]
Grass Carp Stocking				
Total weight (kg)	0.1±0.0 [a]	0.1±0.0 [a]	0.2±0.0 [a]	0.2±0.0 [a]
Mean weight (g)	36.6±8.1 [a]	32.8±10.2 [a]	51.5±6.0 [a]	37.9±1.7 [a]
Harvest				
Total weight (kg)	1.3±0.2 [a]	1.5±0.2 [a]	0.8±0.1 [a]	0.5±0.3 [a]
Mean weight (g)	473.3±46.8 [a]	440.0±17.6 [a]	408.3±59.1 [a]	266.7±16.7 [b]
Daily weight gain (g d^{-1})	2.4±0.3 [a]	2.3±0.1 [a]	2.0±0.3 [a]	1.3±0.1 [b]
Survival (%)	73.3±13.3 [a]	86.7±6.7 [a]	53.3±6.7 [a]	40.0±20.0 [a]
GFY (t ha^{-1} yr^{-1})	0.3±0.0 [ab]	0.3±0.0 [a]	0.2±0.0 [bc]	0.1±0.1 [c]
NFY (t ha^{-1} yr^{-1})	0.2±0.0 [a]	0.3±0.0 [a]	0.1±0.0 [b]	0.1±0.1 [b]
Rohu Stocking				
Total weight (kg)	0.1±0.0 [a]	0.1±0.0 [a]	0.1±0.0 [a]	0.1±0.0 [a]
Mean weight (g)	8.9±3.6 [a]	9.1±3.3 [a]	10.5±2.2 [a]	10.5±4.0 [a]
Harvest				
Total weight (kg)	2.4±0.3 [a]	2.4±0.1 [a]	1.4±0.3 [b]	2.0±0.1 [a]
Mean weight (g)	318.0±40.1 [a]	327.6±11.7 [a]	248.9±10.6 [a]	327.7±36.4 [a]
Daily weight gain (g d^{-1})	1.7±0.2 [a]	1.8±0.1 [a]	1.3±0.1 [a]	1.8±0.2 [a]
Survival (%)	96.7±3.3 [a]	93.3±3.3 [a]	73.3±12.0 [a]	80.0±5.8 [a]

Parameters	Treatment			
	T_1	T_2	T_3	T_4
GFY (t ha^{-1} yr^{-1})	0.5±0.1 [a]	0.5±0.0 [a]	0.3±0.1 [b]	0.4±0.0 [ab]
NFY (t ha^{-1} yr^{-1})	0.5±0.1 [a]	0.5±0.0 [a]	0.3±0.1 [b]	0.4±0.0 [ab]
Mrigal Stocking				
Total weight (kg)	0.05±0.02 [a]	0.04±0.01 [a]	0.06±0.00 [a]	0.06±0.00 [a]
Mean weight (g)	6.3±2.4 [a]	5.6±1.7 [a]	8.3±0.2 [a]	7.3±0.9 [a]
Harvest				
Total weight (kg)	2.2±0.2 [a]	2.4±0.1 [a]	2.2±0.3 [a]	2.7±0.3 [a]
Mean weight (g)	366.0±9.9 [a]	353.2±17.2 [a]	319.5±7.7 [a]	340.0±20.8 [a]
Daily weight gain (g d^{-1})	2.0±0.1 [a]	1.9±0.1 [a]	1.7±0.0 [a]	1.8±0.1 [a]
Survival (%)	80.0±10.0 [a]	86.7±3.3 [a]	90.0±5.8 [a]	100.0±0.0 [a]
GFY (t ha^{-1} yr^{-1})	0.4±0.0 [a]	0.5±0.0 [a]	0.4±0.1 [a]	0.5±0.1 [a]
NFY (t/ ha^{-1} yr^{-1})	0.4±0.0 [a]	0.5±0.0 [a]	0.4±0.1 [a]	0.5±0.1 [a]
Carps Combined				
Gross Fish Yield (t/ha/yr)	4.1±0.1 [a]	4.0±0.2 [a]	3.4±0.1 [b]	3.6±0.3 [ab]
Net Fish Yield (t ha^{-1} yr^{-1})	3.7±0.1 [a]	3.7±0.1 [a]	3.1±0.1 [b]	3.2±0.2 [b]
Survival (%)	79.8±9.1 [ab]	88.8±3.4 [a]	75.2±0.6 [ab]	67.8±7.7 [b]

Table 3. Tilapia recruitment from ponds (100 m^2) in different treatments during the experimental period of 180 days. Mean values with same superscript in the same row are not significantly different ($p<0.05$).

Parameter	Treatments			
	T_1	T_2	T_3	T_4
Mean number (count pond^{-1})	-	798±32 [a]	676±51 [ab]	603±72 [b]
Mean weight (g)	-	8.4±0.5 [a]	10.3±1.8 [a]	11.8±0.8 [a]
Net Fish Yield (t ha^{-1} yr^{-1})	-	1.3±0.1 [a]	1.4±0.2 [a]	1.4±0.2 [a]

Table 4. Combined performance of carps, tilapia and sahar in each treatment. Based on 100 m^2 pond size. Mean values with same superscript in the same row are not significantly different ($p<0.05$).

Parameter	Treatments			
	T_1	T_2	T_3	T_4
Initial Fish Biomass (kg pond^{-1})	1.6±0.1 [a]	1.8±0.3 [a]	2.0±0.2 [a]	2.7±0.6 [a]
Final Fish Biomass (kg pond^{-1})	20.3±0.6 [b]	24.1±1.0 [a]	20.5±1.0 [b]	22.4±1.0 [ab]
Fish Biomass Gain (kg pond^{-1})	18.7±0.6 [b]	22.4±0.8 [a]	18.5±0.8 [b]	19.8±0.5 [b]
Gross Fish Yield (t ha^{-1} yr^{-1})	4.1±0.1[b]	4.8±0.2[a]	4.1±0.2[b]	4.5±0.2[ab]
Net Fish Yield (t ha^{-1} yr^{-1})	3.7±0.1 [b]	4.5±0.2 [a]	3.7±0.2 [b]	4.0±0.1 [b]
AFCR	1.2±0.0 [ab]	1.0±0.0 [b]	1.2±0.1 [ab]	1.4±0.1 [a]
Net Fish Yield including tilapia recruits (t ha^{-1} yr^{-1})	3.7±0.1[c]	5.8±0.3[a]	5.1±0.1[b]	5.4±0.2[ab]

Table 5. Overall mean and ranges of water quality parameters in each treatment.

Parameter	Treatments			
	T_1	T_2	T_3	T_4
Temperature (°C)	27.3±0.1 (20.8-31.5)	27.3±0.1 (20.7-31.7)	27.3±0.1 (20.7-31.9)	27.3±0.1 (20.7-31.7)
pH	7.5 (7.2-8.7)	7.6 (7.2-8.9)	7.5 (7.1-9.0)	7.4 (7.1-8.9)
DO (mg L^{-1})	2.9±0.8 (0.6-7.2)	3.2±0.7 (0.8-7.1)	2.7±0.4 (0.3-6.7)	1.9±0.5 (0.3-0.5)
Secchi depth (cm)	27.8±1.7 (16.7-45.7)	25.9±0.8 (15.3-42.2)	28.4±2.5 (15.7-42.7)	31.0±2.1 (14.7-50.3)
Total alkalinity (mg L^{-1})	190.6±7.1 (112.2-283.1)	183.2±9.1 (130.1-267.5)	190.6±9.2 (102.3-276.8)	202.0±9.0 (104.4-292.8)
TAN (mg L^{-1})	0.37±0.01 (0.09-0.73)	0.39±0.04 (0.08-0.82)	0.34±0.03 (0.08-0.70)	0.36±0.01 (0.09-0.69)
Nitrite-N (mg L^{-1})	0.25±0.04 (0.13-0.50)	0.22±0.03 (0.13-0.38)	0.23±0.02 (0.13-0.46)	0.21±0.02 (0.13-0.40)
SRP (mg L^{-1})	0.42±0.01 (0.24-0.64)	0.44±0.02 (0.21-0.73)	0.38±0.02 (0.20-0.58)	0.45±0.06 (0.22-0.78)
Chlorophyll-a (mg m^{-3})	198.3±24.0 (21.4-340.4)	156.0±3.0 (34.7-242.4)	179.9±13.9 (47.7-295.8)	155.8±9.2 (33.6-271.2)

Table 6. Comparative economic analysis (in NRs) for ponds in each treatment during the experiment. Mean values with same superscript in the same row are not significantly different ($p<0.05$). 1 US$=73 NRs.

Parameter	Treatments			
	T_1	T_2	T_3	T_4
Gross Return				
Adult Nile tilapia	-	818.9±63.3 ^{a}	630.4±103.7 ^{a}	831.9±65.5 ^{a}
Sahar	-	-	45.6±7.8 ^{b}	92.3±1.7 ^{a}
Carps	3630.6±92.3 ^{a}	3573.1±152.2 ^{a}	2991.7±132.8 ^{b}	3153.7±226.1 ^{ab}
Nile tilapia recruits	-	798.3±31.8 ^{a}	676.0±51.1 ^{ab}	603.3±72.3 ^{b}
Total	3630.6±92.3 ^{c}	5222.1±296.5 ^{a}	4343.5±223.4 ^{b}	4681.3±212.3 ^{ab}
Variable Cost				
Nile tilapia (fingerlings)	-	60.5±0.5 ^{a}	60.5±0.5 ^{a}	60.5±0.5 ^{a}
Sahar (fingerlings)	-	-	26.2±0.6 ^{b}	51.5±1.5 ^{a}
Carps (fingerlings)	199.4±1.8 ^{a}	199.4±1.8 ^{a}	199.4±1.8 ^{a}	199.4±1.8 ^{a}
Lime	100.0±0.0 ^{a}	100.0±0.0 ^{a}	100.0±0.0 ^{a}	100.0±0.0 ^{a}
Feed	437.8±10.6 ^{a}	450.3±34.1 ^{a}	443.6±45.3 ^{a}	551.4±40.3 ^{a}
DAP	173.3±0.0 ^{a}	173.3±0.0 ^{a}	173.3±0.0 ^{a}	173.3±0.0 ^{a}
Urea	155.7±0.0 ^{a}	155.7±0.0 ^{a}	155.7±0.0 ^{a}	155.7±0.0 ^{a}
Cost of working capital	106.6±1.2 ^{b}	113.9±3.5 ^{b}	115.7±4.6 ^{b}	129.4±4.1 ^{a}
Total variable cost	1172.7±13.5 ^{b}	1253.1±38.2 ^{b}	1273.1±50.1 ^{b}	1423.5±45.4 ^{a}
Gross margin (in '000) (NRS $pond^{-1}$)	2.5±0.1 ^{c}	4.0±0.3 ^{a}	3.1±0.2 ^{bc}	3.3±0.2 ^{b}
Gross margin (in '000) (NRS ha^{-1} $year^{-1}$)	491.6±15.7 ^{c}	793.8±52.2 ^{a}	614.1±46.5 ^{bc}	651.5±39.2 ^{b}

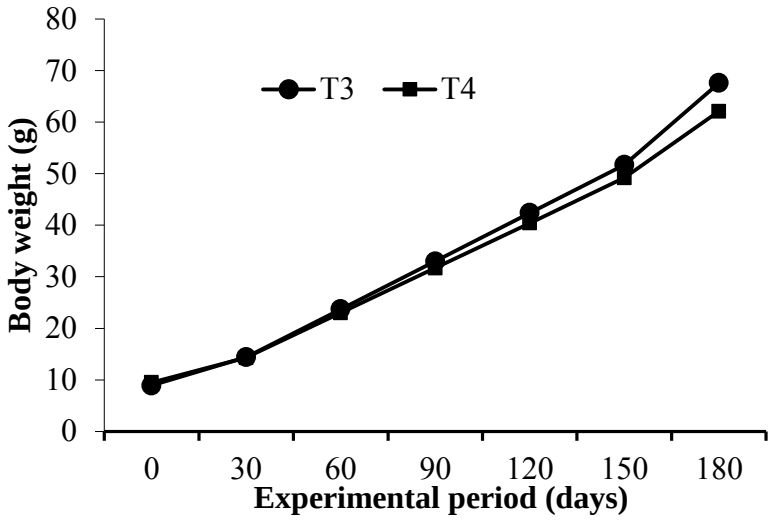


Figure 1. Growth trend of sahar in each treatment during the experiment period of 180 days (25 February to 26 August, 2011).

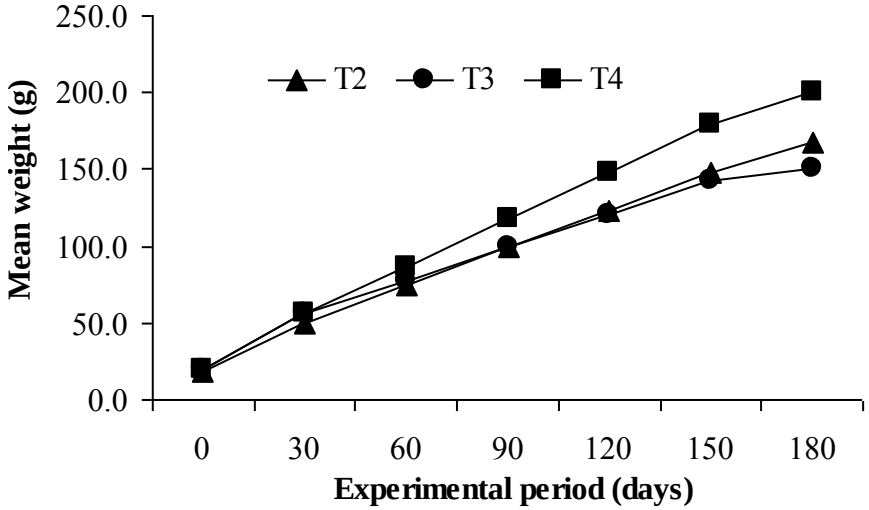


Figure 2. Growth trend of Nile tilapia in each treatment during the experiment periodof 180 days (25 February to 26 August, 2011).

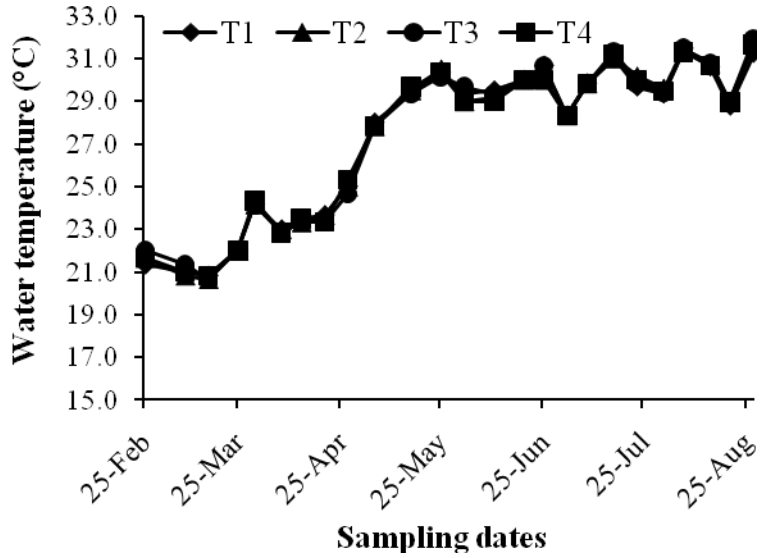


Figure 3. Weekly mean temperature (°C) of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

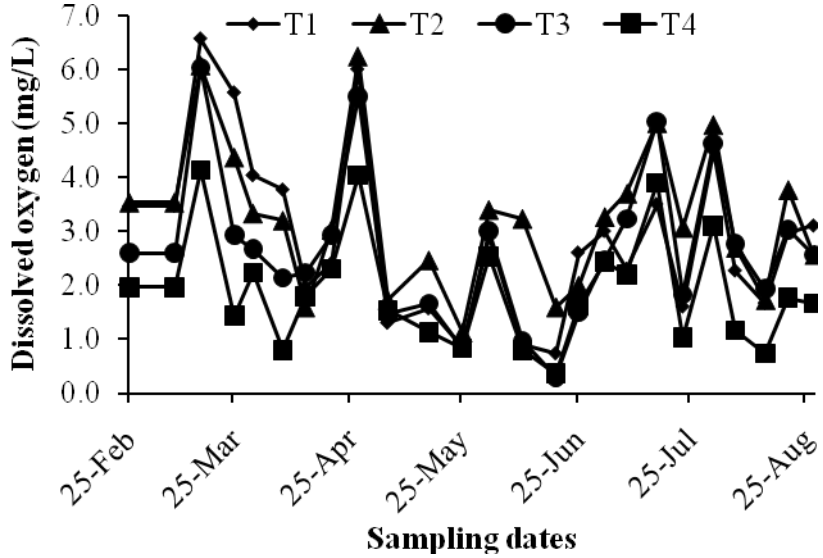


Figure 4. Weekly mean dissolved oxygen (mg L^{-1}) of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

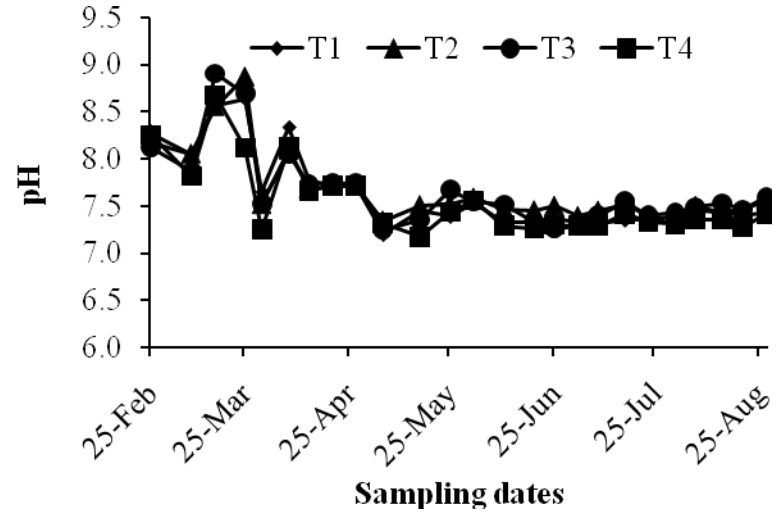


Figure 5. Weekly mean pH of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

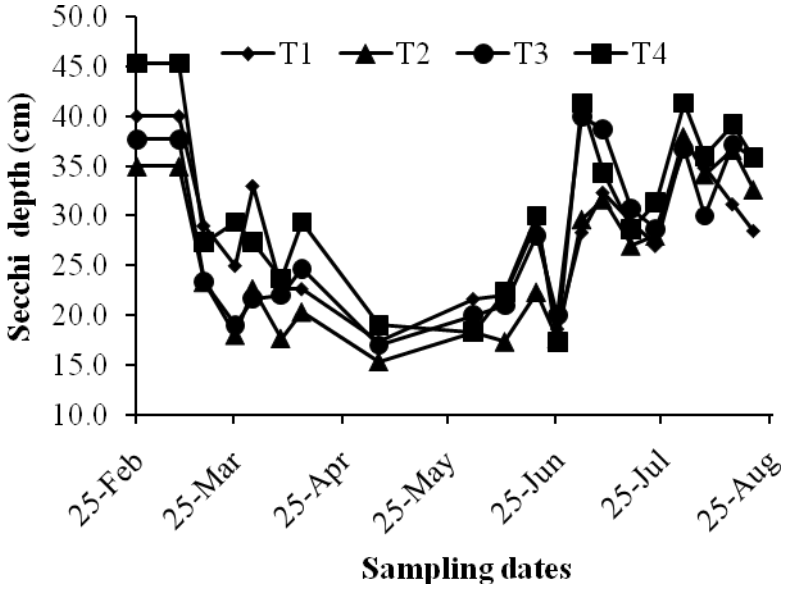


Figure 6. Weekly mean secchi depth (cm) of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

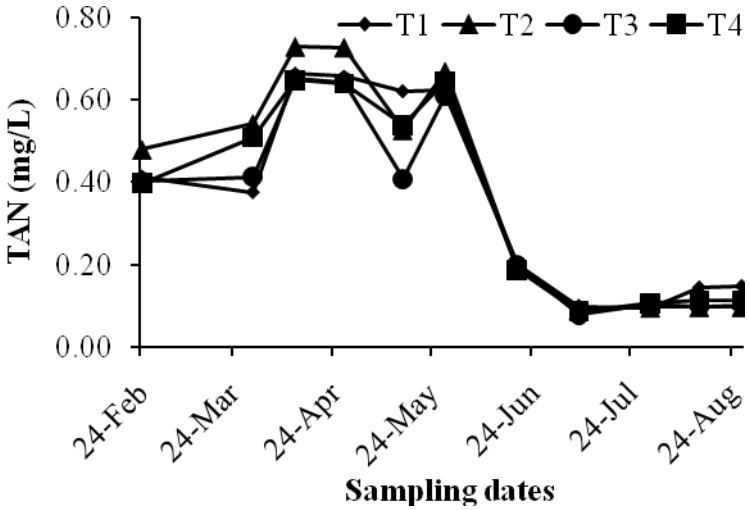


Figure 7. Fortnightly mean total ammonium nitrogen (TAN) (mg L^{-1}) of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

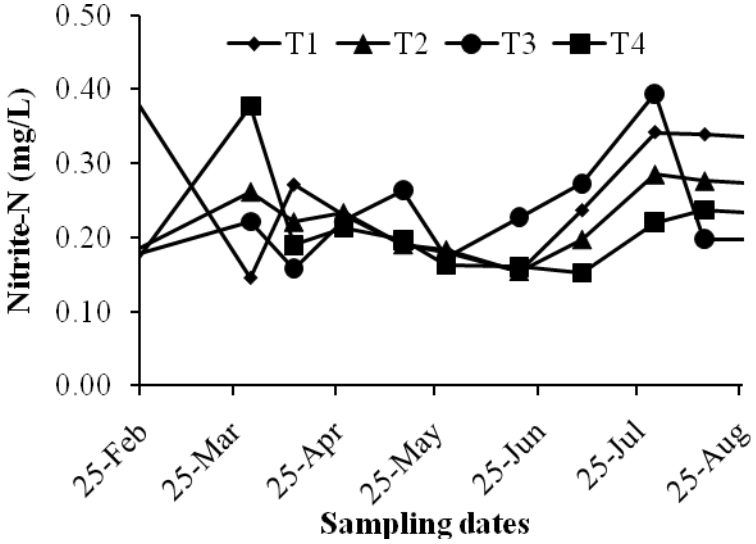


Figure 8. Fortnightly mean nitrite ammonium nitrogen (Nitrite-N) (mg L^{-1}) of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

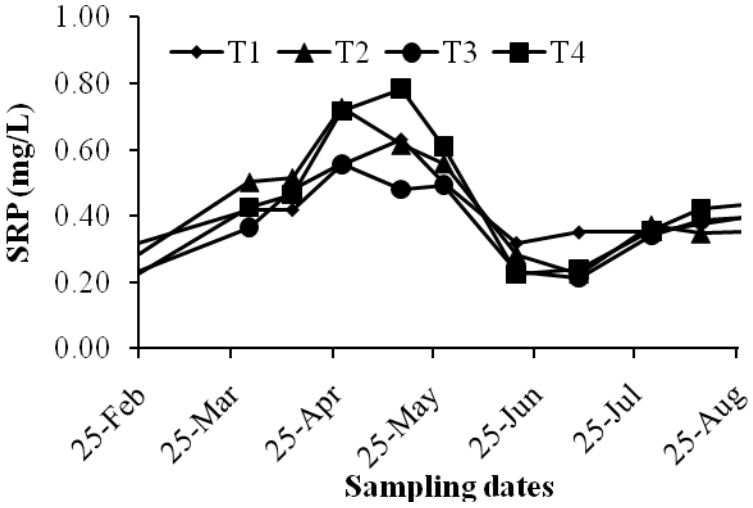


Figure 9. Fortnightly mean soluble reactive phosphorous (SRP) (mg L^{-1}) of pond water at 0600–0800 h in each treatment during the experimental period (25 February to 26 August, 2011).

Study on the Effectiveness of a Pond-Based Recirculating System for Shrimp Culture

Production System Design and Best Management Alternatives/Experiment/09BMA04UM

Lai Qiuming
Hainan University
Haikou City, China

Yuan Derun
Network of Aquaculture Centres in Asia-Pacific
Bangkok, Thailand

Liu Liping
Shanghai Ocean University
Shanghai, China

James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

ABSTRACT

An experiment was conducted in four earthen ponds (about 0.35 ha each) at Haoshideng Shrimp Farm, Hainan Province, China from March 2010 to July 2011 to test efficiency of a pond based recirculating shrimp culture system equipped with a water treatment facility consisting of a screen drum filter and a foam fractionating unit. Shrimp post-larvae were stocked at 100 shrimp m^{-2} and cultured in replicates in a recirculating system and an open system with 15% weekly water exchange. The experiment was repeated for twice in two years in the same ponds with similar weather conditions. Shrimp survival rate ranged from 59 - 64% and shrimp yield ranged from 8081 – 9931 kg ha^{-1}, with the recirculating system having significantly higher shrimp survival rate and yield than the open system. When pond water passed through the treatment facility, TSS, COD, TAN, TKN and TP in water were significantly reduced and DO increased. Biweekly water sampling and analyses however did not show any significant differences in water quality for ponds of each system, indicating the water treatment facility had a waste nutrient removing capacity equivalent to 15% water exchange weekly. The recirculating system was more environmentally friendly without effluent discharge during production, and produced higher yields than the exchange system.

INTRODUCTION

Worldwide shrimp production increased rapidly from 71,432 metric tons in 1980 to 3,399,105 metric tons in 2008 with a total value of USD 14.3 billion (FAO, 2010). Despite continuously increasing market demand, further development and expansion of shrimp is constrained by environmental concerns associated with discharge of effluents and dispersal of solid wastes to the environment. This has renewed interest in recirculating systems due to their perceived advantages, including reduced use of water, greater control of the culture environment, reduced or eliminated use of antibiotics and hazardous chemicals, and close to zero discharge of effluents. Attempts have been made to develop recirculating shrimp culture systems using indoor tanks or raceways (Wyk et al., 1999) and outdoor ponds (Lin, 1995; Neori et al., 1996; Shpigel and Neori, 1996; Neori and Shpigel, 1999; Jones et al., 2001). Commercial-scale aquaculture has become possible using these systems and practices. However, a tank- or raceway-based

indoor recirculating system is technically sophisticated, economically expensive, and may be impractical for the majority of small-scale shrimp farmers in Asia. On the other hand, integrated pond-based recirculating systems require a large percentage of the farm area to culture treatment organisms such as seaweeds, bivalves, and filter-feeding fish, which limits their application. Combining shrimp pond culture with efficient waste treatment components similar to those used in indoor tank systems but with limited complexity of operation and maintenance may be a feasible alternative.

Hainan province, the only tropical area in China, is one of the major shrimp production areas. The environmental impact of shrimp culture has become a serious concern. Thus, this experiment was conducted to test a pond-based recirculating system at Haoshideng Shrimp Farm in Hainan province to eliminate effluent discharge and use solid wastes as fertilizer for coconut trees.

The specific objectives were:
1. To evaluate effectiveness of combining a screen drum filter and a foam fractionating unit to remove solid wastes and improve water quality of shrimp culture ponds;
2. To compare water quality parameters in recirculating and open shrimp culture ponds;
3. To compare overall production performance between recirculating and open shrimp culture ponds.

MATERIALS AND METHODS

The experiment was conducted in four earthen ponds of 0.33 ha at Haoshideng Shrimp Farm, at the northern tip of Hainan province, China from March 2010 to July 2011. Drainage pipes were located in the center of ponds and pond bottoms and dikes were lined with plastic. Pond water depth was maintained at about 1.3 m and four paddle wheels were installed in each pond to provide aeration. White shrimp post-larvae were stocked at 100 pieces m^{-2} and fed with commercial pellets. The feeding rate was determined using feeding trays.

The four shrimp ponds were randomly divided into treatment and control groups, with two ponds in each group. Water in ponds of the treatment group flowed through a screen drum filter and a foam fractionating unit before recirculating back to the ponds (Figure 1). Water in ponds of the control group was exchanged weekly at 15%. Unfortunately both ponds and water were limited, constraining the number of replicates. To overcome this shortfall, the experiment was repeated for twice in two years with the same ponds and facilities and similar weather conditions.

Water quality parameters in ponds were measured once every two weeks. Temperature, DO, and pH at dawn were measured in situ using a DO meter (YSI Model DO-100) and a pH meter (YSI Model pH-200). Secchi disk visibility, water depth, and salinity were measured in situ at 0900 h using a Secchi disk and a hand-held refractometer. Pond water was sampled once at 0900 h for analyses of specific reactive phosphorus (SRP), total phosphorus (TP), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total ammonia nitrogen (TAN), total Kjedahl nitrogen (TKN), chemical oxygen demand (COD), chlorophyll α, total suspended solid (TSS), and total alkalinity following standard methods (APHA et al., 1995).

For ponds of the treatment group, water was also sampled once every two weeks before and after circulation through the screen drum filter and foam fractionating unit for water quality analysis. The same parameters and methods were used as above.

Data from two replicate experiments were pooled together. Production and pond water quality parameters were analyzed statistically by analysis of variance (Steele and Torrie, 1980), while paired t-test was used to compare water quality parameters before and after water treatment in the recirculating system. SPSS (version 16.0) statistic software package (SPSS Inc., Chicago, USA) was used for statistical analyses. Differences were considered significant at an alpha level of 0.05. Means were given with ± standard error (S.E.). Percentage data were transferred to arcsine before analysis but presented in the original form.

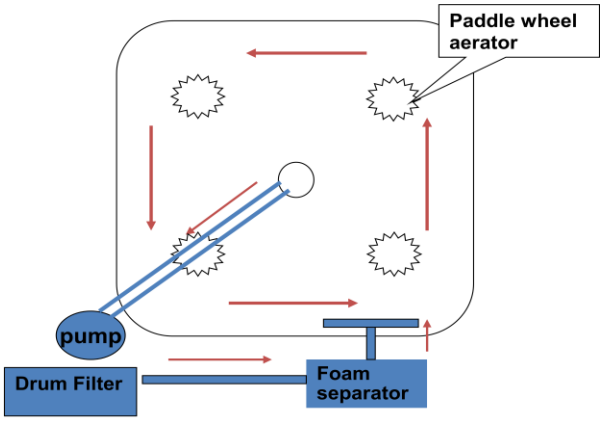


Figure 1. The work flow chart of the recirculating system for shrimp culture

RESULTS

The technical details of the recirculating system (Table 1) included a screen drum filter and a foam fractionating unit designed for a pond area about 0.35 ha. The screen drum filter separated suspended solids with particle size larger than 700 µm, through physical filtration while the foam fractionating unit further partially separated smaller suspended particles and some large organic molecules by injecting air bubbles and foam formation. Testing of water drained from the central drainage pipe before treatment, recirculating back to ponds after treatment, and waste water collected from the treatment units showed a significant decrease of total suspended solids, COD, total ammonia nitrogen, TKN, TP after water treatment ($P<0.05$), while nitrite and nitrate nitrogen trends were not significant (Table 2). DO was significantly increased after water flowing through the treatment unit.

Table 1. Mechanical specifications of a screen drum filter and a foam fractionating unit used in this experiment

Parameters	Specifications
Screen drum filter	
Motor power (Kw)	0.5
Drum length (m)	1.2
Drum diameter (m)	0.7
Screen mesh size (µm)	700
Drum rotation speed (revolution minute^{-1})	10
Water flow-through rate (m^3 h^{-1})	25
Operation time (h day^{-1})	24
Foam fractionating unit	
Power (Kw)	1.0
Unit volume (m^3)	1.5
Water flow-through rate (m^3 h^{-1})	25
Flow system	1

Table 2. Water quality change before and after water passed through the screen drum filter and the foam fractionating unit in recirculating ponds of the experiment.

Parameters	Before	After	Mixed waste water
DO (mg L^{-1})[1]	4.80 ±0.51	5.23 ±0.42	-----
pH	7.94±0.21	8.08±0.13	7.41±0.24
TSS (mg L^{-1})[1]	0.0412±0.0243	0.0346±0.0204	1.3289± 0.4159
COD (mg L^{-1})[1]	7.9536±2.7042	6.9161±2.4644	75.2052±19.5465
Nitrite (mg L^{-1})	0.2036±0.2649	0.2023±0.2642	0.5002±0.5616
Nitrate (mg L^{-1})	0.3054±0.4154	0.3046±0.4155	0.7155±0.7652
TAN (mg L^{-1})[1]	0.2598±0.2159	0.2356±0.1984	2.0654±1.6868
TKN (mg L^{-1})[1]	4.4895±2.3567	3.9385±2.0335	41.4196±18.1711
TP (mg L^{-1})[1]	0.6226±0.1190	0.5432±0.1650	4.1941±1.5894

Parameters followed by a superscript had significant differences between before and after values ($p<0.05$).

Shrimp grew better in treatment ponds than control ponds. Shrimp in treatment ponds had significantly higher survival rate, daily weight gain, and yield per unit area than shrimp in control ponds ($P<0.05$). FCR was not significantly different between treatment and control ponds (Table 3).

Table 3. Growth and production performance of white shrimp in ponds in each treatment.

Parameter	Open system	Recirculating system
Stocking		
Pond size (ha)	0.34±0.01	0.37±0
Biomass (kg $pond^{-1}$)	10.2±0.17	10.95±0.09
Number of post-larvae (No. $pond^{-1}$)	340,000±5,774	365,000±2,887
Density (shrimp m^{-2})	100±0	100±0
Harvest		
Growing period (days)	99±1.73	98±1.73
Shrimp (No. $pond^{-1}$)	199,609±6,972	233,761±4,974
Biomass (kg pond-^{1})	2747.25±44.97	3624.25±44.05
Mean weight (g $shrimp^{-1}$)	13.81±0.46	15.54±0.5
Total weight gain (kg $pond^{-1}$)	2737.05±44.82	3613.3±44.05
Performance		
Daily weight gain (g shrimp-^{1} day^{-1})	0.14±0[b]	0.16±0[a]
Yield (kg ha^{-1})	8081.02±59.32[b]	9931.1±137.68[a]
Survival (%)	0.59±0.02[b]	0.64±0.01[a]
FCR	1.29±0.04	1.21±0.03

Values in a row with different superscripts were significantly different between pond types ($p < 0.05$).

There were also no significant differences in water quality parameters between treatment and control ponds (Table 4).

Table 4. Values of water quality parameters measured during the experiment.

Parameter	Open system	Recirculating system
Mean Values		
Secchi disk visibility (cm)	48.03±0.89	48.91±3.06
Water depth (cm)	132.69±0.28	133.47±1.03
Temperature (^{o}C)	29.01±0.09	28.85±0.04
DO (mg L^{-1})	6.72±0.23	6.73±0.04
Salinity (PPT)	30.11±0.08	30.06±0.3
pH	8.1±0.04	8.03±0.02
SRP (mg L^{-1})	0.08±0.01	0.09±0.01
TP (mg L^{-1})	0.72±0.1	0.69±0.07
Nitrite (mg L^{-1})	0.15±0.05	0.16±0.02
Nitrate (mg L^{-1})	0.28±0.01	0.31±0.05
TAN (mg L^{-1})	0.17±0.01	0.22±0.02
TKN (mg L^{-1})	4.15±0.26	4.38±0.43
COD (mg L^{-1})	8.18±0.17	7.95±0.4
Chl-a (mg L^{-1})	265.05±27.08	233.57±33.39
TSS (g L^{-1})	0.05±0.01	0.04±0.01
Alkalinity (mmol dm^{-3})	3.04±0.08	3.1±0.06
End values		
Secchi disk visibility (cm)	32.5±2.5	29.5±2.1
Water depth (cm)	136.25±1.25	135.5±1.66
Temperature (^{o}C)	32±0.06	31.96±0.05
DO (mg L^{-1})	7.29±0.59	7.95±0.55
Salinity (PPT)	30.9±0.25	31.03±0.09
pH	7.55±0.22	7.63±0.11
SRP (mg L^{-1})	0.09±0.04	0.05±0.03
TP (mg L^{-1})	0.73±0.06	0.83±0.09
Nitrite (mg L^{-1})	0.28±0.14	0.24±0.05
Nitrate (mg L^{-1})	0.26±0.12	0.11±0.05
TAN (mg L^{-1})	0.25±0.07	0.29±0.05
TKN (mg L^{-1})	4.5±0.48	5.16±0.47
COD (mg L^{-1})	11.1±0.21	11.51±0.03
Chl-a (mg L^{-1})	298.52±57.81	546.9±128.94
TSS (g L^{-1})	0.04±0.01	0.06±0.02
Alkalinity (mmol dm^{-3})	2.9±0.15	3±0.08

DISCUSSION

Water exchange was traditionally used to maintain pond water quality and reduce waste accumulation in intensive shrimp culture (Fast, 1991). It however carries the risk of introducing diseases with inflow water, and could cause drastic changes in pond water quality (Hopkins et al., 1995). In this experiment, 15% of the pond's volume was exchanged weekly in control ponds. This water exchange maintained water quality at similar levels to the treatment ponds, but probably was a stress factor which resulted in lower shrimp survival and production in control ponds.

This experiment did not reveal any significant differences in nutrient contents in pond water between the open system and the recirculating system. This indicated that the water treatment facility had a nutrient removing capacity equivalent to a 15% water exchange weekly.

Environmental pollution has been associated with intensive shrimp pond culture since early 1910s (Pruder, 1992; Phillips et al., 1993; Boyd and Clay, 1998; Fast and Menasveta, 2000). This is primarily caused by the nature of the systems in which only small portion of the nutrient input is harvested through shrimp, while the majority is discharged into natural waters (Briggs and Funge-Smith, 1994; Lin, 1995; Teichert-Coddington et al., 2000; Jackson et al., 2003). Sophisticated waste treatment systems are often costly and impractical, especially for small scale shrimp farms which are still the dominant types in major shrimp producing countries such as China. This experiment demonstrated that installation of simple waste water treatment facilities such as screen drum filters and foam fractionators is effective to remove wastes produced in shrimp ponds without effluent discharge to surround environment during culture. In addition, shrimp survival and production was actually improved in the recirculating system. The recirculating system was therefore more environmentally friendly and more productive than the traditional open system with period water exchange.

ANTICIPATED BENEFITS

Whiteleg shrimp (*Litopeneaus vannamei*) is the most important shrimp species cultured throughout the region. Testing and demonstration of the proposed pond-based recirculating system will lead to further development, fine tuning and extension of recirculating systems which are suitable to the majority of small scale shrimp farms in Asia. This will reduce environmental impacts of intensive shrimp culture and improve its sustainability. Since shrimp imports are dominated by the U.S., better knowledge of sustainable shrimp culture will benefit NGOs like World Wildlife Fund, as well as private citizens and markets concerned with reducing the environmental footprint of shrimp culture.

LITERATURE CITED

APHA, AWWA, and WPCF, 1995. Standard Methods for the Examination of Water and Wastewater, 20th Edition. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, D.C.

Boyd, C.E., and J.W. Clay, 1998. Shrimp aquaculture and the environment. Scientific American, June 1998:58-65.

Briggs, M.R.P., and S.J. Funge-Smith, 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. Aquaculture and Fisheries Management, 25:789-811.

Fast, A.W., 1991. Marine shrimp pond grow-out conditions and strategies: A review and prognosis. Reviews in Aquatic Sciences, 3:357-400.

Fast, A.W., and P. Menasveta, 2000. Some recent issues and innovations in marine shrimp pond culture. Reviews in Fisheries Science, 8:151-233.

FAO, 2008. Fishstat, Fisheries Department, Food and Agriculture Organization, Rome.

Jackson, C., N. Preston, P., J. Thompson, and M. Burford, 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture, 218: 397-411.

Jones, A.B., W.C. Denniso, and N.P. Preston, 2001. Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study. Aquaculture, 193:155-178.

Lin, C.K., 1995. Progression of intensive marine shrimp farm in Thailand. In: C.L. Browdy and J.S. Hopkins (Editors), Swimming Through Troubled Water, Proceedings of the Special Session on Shrimp Farming, Aquaculture'95. World Aquaculture Society, Baton Rouge, Louisiana, pp. 13-22.

Neori, A., M.D. Krom, S.P. Ellner, C.E. Boyd, D. Popper, R. Rabinovitch, P.J. Davidson, O. Divr, D. Zuber, M. Ucko, D. Angel, and H. Gordin, 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. Aquaculture, 141:183-199.

Neori, A., and M. Shpigel, 1999. Using algae to treat effluents and feed invertebrates in sustainable integrated mariculture. World Aquaculture, June 1999:46-49.

Pruder, G. D., 1992. Marine shrimp pond effluent: characterization and environmental impact. In: J. Wyban (Editor). Proceedings of Special Session on Shrimp Farming, World Aquaculture Society, Boton Rouge, LA USA., pp. 187-190.

Phillips, M.J., C.K. Lin, and M.C. Beveridge, 1993. Shrimp culture and the environment: lessons from the world's most rapidly expanding warmwater aquaculture sector. In: R.S.V. Pullin, H. Rosenthal, and J.L. Maclean (Editors). Environment and Aquaculture in Developing Countries. ICLARM Conference Proceedings 31, Manila, Philippines. pp. 179-191.

Shpigel, M., and A. Neori, 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. Aquaculture Engineering, 15:313-326.

Steele, R.G.D., and J.H. Torrie, 1980. Principles and Procedures of Statistics : A Biometrical Approach. McGraw Hill, New York, USA 533 pp.

Teichert-Coddington, D.R., D. Martinez, and E. Ramirez, 2000. Partial nutrient budgets for semi-intensive shrimp farms in Honduras. Aquaculture, 190:139-145.

Wyk, P.V., M. Davis-Hodgkins, R. Laramore, K.L. Main, J. Mountain and J. Scarpa, 1999. Farming Marine Shrimp in Recirculating Freshwater System. Harbor Branch Oceanographic Institute, Florida, U.S., 220 pp.

Development of Indoor Recirculating Culture Systems for Intensive Shrimp Production in China

Production System Design and Best Management Alternatives/Experiment/09BMA05UM

Part 1. Objectives 1–3

Jiang Min, Liu Liping, Dai Xilin, Yu Gending, Qu Rui, Li Shikai
Shanghai Ocean University
Shanghai, China

James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

ABSTRACT

Indoor recirculating ponds, traditional ponds and eco-culture ponds were evaluated for water quality and *Litopenaeus vannamei* culture in 2010 and 2011. pH changed more dramatically in outdoor ponds. Nutrient content such as TN, TP, NO_2-N, NO_3-N, TAN and PO_4-P were lowest in eco-culture ponds, then in indoor RAS, and highest in traditional outdoor ponds. Nutrient budget analysis showed that both nitrogen and phosphorus gain to ponds occurred mostly from feed (nitrogen: 94.95% in indoor RAS, 85.21% in traditional ponds and 88.06% in eco-culture ponds; phosphorus: 97.06%, 92.34% and 94.87%, respectively), while 34.24% of nitrogen and 16.84% of phosphorus were retained in shrimp from indoor RAS, 31.02% N and 13.21% P were retained in shrimp from traditional outdoor ponds, and 35.73% and 15.25% for eco-culture ponds. Phosphorus was more important than nitrogen causing algae blooms during shrimp culture. Good water quality is essential, but not the only factor that affects shrimp production.

INTRODUCTION

Shrimp is a favored aquatic product around the world since it has high protein, low fat and is rich in nutrition. Marine shrimp has a wide range of adaptability to salinity and can be cultured in salt, brackish, or fresh water. China's shrimp farming industry has made remarkable achievements in the last two decades. In 1993, shrimp farming was severely damaged by outbreak of epidemic diseases, but it recovered, and shrimp production increased significantly. This increase was caused by several new technologies, such as the replacement of cultured species, structural transformation of the pond, and disinfection of rearing water.

In recent years, culturing shrimp has been widely developed in Shanghai and adjacent provinces. Traditional aquaculture models are still being applied throughout China, which means high density, high input, high yield, and high water exchange rate, with much drug use and high consumption of energy. These traditional models will not prevent outbreak of diseases and cause water pollution by discharging of wastewater rich in nitrogen, phosphorus, and organic matter to surrounding rivers or lakes. The damaged water ecosystems then cause further disease outbreaks, which in turn threaten human health and food safety.

Intensive fish farming has a long history in many countries (Sung-Koo et al., 2000). Production by a Danish aquaculture company was from 100-300 kg/m^3. Although there is noticeable gap between China and other developed countries in facilities and techniques (Chen 1998), indoor intensive aquaculture has

also been explored around China (Ying, 2001). Water treatment equipment and technologies have been developed and successful systems have been introduced to culturists. Indoor intensive aquaculture technologies have been used in culturing abalone, Atlantic turbot *(Scophthalmus maximus)*, and flounder in Shandong, Liaoning, and other provinces (Chang-fa, 2002).

Indoor intensive aquaculture has been developed worldwide. Recycling intensive shrimp farming has succeeded in Hawaii, Florida, Texas, and other places, and may produce approximately 5-10 kg shrimp per cubic meter of water in three months. In indoor intensive shrimp aquaculture, the most important thing is to control water quality and micro-organisms within desirable levels.

Considering the cost of infrastructure construction, relatively high electricity use, and need for highly educated or at least high skilled farmers, people in China are uncertain whether to develop indoor RAS or outdoor eco-culture systems. In this study, three different systems for *Litopenaeus vannamei* culture were studied to discuss their feasibility and effect.

METHODS

The research was originally designed to be conducted at Shanghai Bluesea Aquatech Co., Ltd, Fengxian District, Shanghai, China. In 2010, the infrastructure construction for greenhouse was delayed due to local policy for land use planning, so our study on an intensive indoor recirculating system (Figure 1) for *Litopenaeus vannamei* culture was carried out at Langxia's Special Cultivation Co., Jinshan District, Shanghai. At the same time, 22 outdoor ponds with traditional culture technologies (Figure 2) and 3 eco-culture ponds with special water quality control technology (Figure 3) were monitored for survival rate of shrimp and water quality. According to the results of shrimp production in 2010, we continued the study only on 17 eco-culture ponds in Bluesea Co. in 2011.

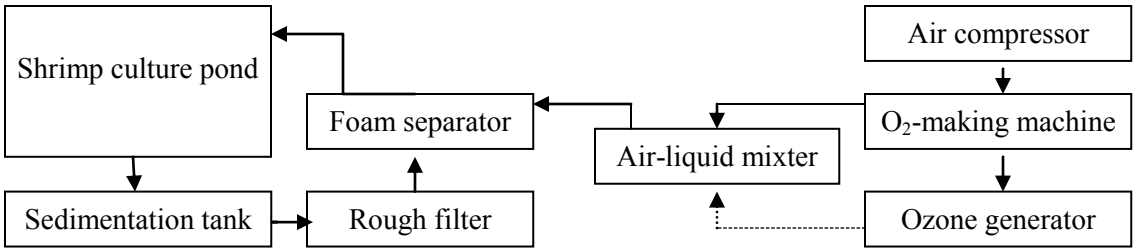


Figure 1. Flow diagram of our intensive indoor recirculating system.

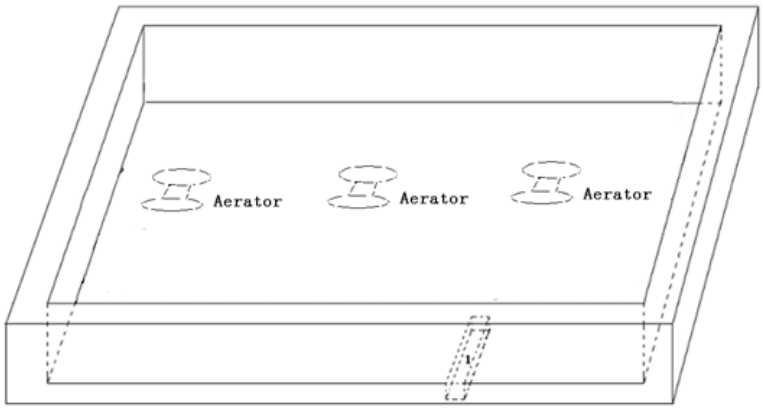


Figure 2. Schematic of a traditional outdoor pond.

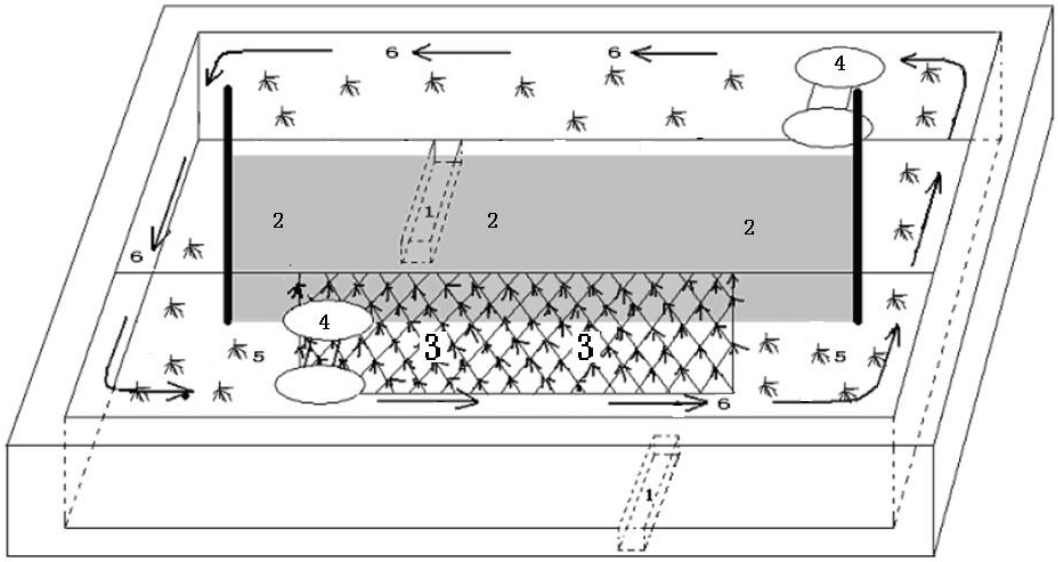


Figure 3. Schematic of our outdoor eco-culture pond. Characteristics included: 1-discharge ditch; 2-canvas divider; 3-water purifying net; 4-aerator; 5-aquatic plants; 6-water flow.

The research was accomplished from 2010 to 2011. Table 1 shows the basic information on facilities and shrimp culture methods for the experimental ponds. Commercial feed was used for the whole culture period. In 2010, all post-larvae of *Litopenaeus vannamei* were bought from Hainan. In 2011, shrimp in 12 ponds were from Hainan Province, while shrimp for the other 5 ponds were nursed by Shanghai Bluesea Aquatech Co., Ltd.

Table 1. Basic conditions and shrimp culture methods for the experimental ponds.

Research year	**Pond type**	**Location**	**Ponds number**	**Pond size**	**Facilities**	**Shrimp density**	Remarks
2010	Indoor recirculating system	Jinshan District	2	700m^2	Settling tank, rough filter, foam separator combined sometimes with ozone treatment constituted the water treatment unit. In each pond, there was a canvas divider (37.65m×1.70m), 6 aerators and several water purifying nets.	342 ind/m^2	One crop June-Sept.
2010	Outdoor traditional pond	Fengxian District	11 ponds A 11 ponds B	A:2855±167m^2 B: 3864±171m^2	Three aerators of 1.5 KW were used in each pond.	85.5±6.0 ind/m^2	Two crops May- July July-Sept.
2010	Outdoor eco-culture pond	Fengxian District	3	550 m^2	Each pond has a piece of canvas, 2 aerators, several water purifying nets, air stripping tubes and aquatic plants	200 ind/m^2	One crop July-Sept.
2011	Outdoor eco-culture pond	Fengxian District	17	550 m^2	Each pond has a piece of canvas, 2 aerators, several water purifying nets and air stripping tubes and aquatic plants	112.5 ind/m^2	One crop July-Sept.

Water samples were collected at the depth of 0.5m, and parameters including pH, dissolved oxygen (DO), Transparency (SD), suspended solid (SS), total organic carbon (TOC), biological oxygen demand (BOD_5), chemical oxygen demand (COD_{Mn}), nitrite (NO_2-N), total ammonia (TAN), nitrate-nitrogen (NO_3-N), total nitrogen (TN), phosphate phosphorus (PO_4-P), and total phosphorus (TP) were measured. Table 2 shows the analytic methods used for these water qualities.

Table 2. Water quality parameters and methods.

Parameters	Method	Parameters	Method
SD	Secchi disc	NO_3-N	DIONEX IC1500
pH	WTW pH330	NO_2-N	DIONEX IC1500
DO	YSI ProPlus	TAN	Nessler's reagent spectrophotometry
SS	Gravimetric method	TN	Alkaline potassium persulfate digestion-UV spectrophotometric method
TOC	TOC-VCPH	TP	Ammonium molybdate spectrophotometric method after alkaline potassium persulfate digestion
COD_{Mn}	Alkaline Permanganate method	PO_4-P	Ammonium molybdate spectrophotometric method
BOD_5	HACH BODTrak™	Chl.a	Acetone extraction spectrophotometric method

In the indoor recirculating system, sediment samples were collected from the drain that was in the middle of the eco-culture pond. In outdoor ponds, sediment was collected from the pond bottom after harvest. TN and TP were analyzed after samples had been mixed and dried. Feed samples were also analyzed for TN and TP.

In 2010, shrimp samples were taken from 22 outdoor ponds with traditional culture technologies and 3 eco-culture ponds every two weeks. The white spot syndrome virus (WWSV), taura syndrome virus (TSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) were monitored. At the same time, the total number of culturable heterotrophic bacteria, and vibrio in pond water were analyzed.

RESULTS AND DISCUSSION

Outdoor ponds had a wider range of pH values and reached higher pH levels compared to indoor ponds (Tables 3-5). DO was not measurably different among the three pond different types.

Technologies used in indoor RAS and eco-culture ponds showed obvious effects on control of water quality. Overall, the concentrations of TAN, NO_2-N, NO_3-N, TN, PO_4-P, and TP in eco-culture ponds were much lower than those in traditional ponds. The indoor recirculating system also had lower concentrations than outdoor traditional ponds, although type b (larger outdoor ponds) had relatively similar water quality to the indoor system.

Both the indoor RAS and eco-culture ponds used aerators and canvas to manage water flow, and provided water purifying nets for attachment of microorganisms. Due to the relatively weak light conditions, aquatic plants did not grow well in the indoor RAS, so they were removed from the ponds soon after culture began. In outdoor eco-culture ponds, plants grew quite well and absorbed inorganic nutrients which caused lower levels of dissolved nitrogen and phosphorus compared to indoor RAS.

In eco-culture ponds, no water was discharged directly to the adjacent environment during the culture period and new water was added only to make up for evaporation loss. In the traditional outdoor ponds, water was not usually discharged if the water quality was controllable. However, in 2010, the water quality in type-a ponds decreased dramatically after the first crop of shrimp, so all water was exchanged.

Table 3. Water quality measurements for the indoor recirculating ponds.

Variable	Pond 1		Pond 2	
	Range	$\bar{x} \pm s$	Range	$\bar{x} \pm s$
pH	7.50-8.36	7.92±0.33	7.50-8.59	7.96±0.38
DO (mg/L)	3.70-8.00	6.43±1.21	3.78-8.42	6.37±1.49
TAN (mg/L)	0.231-1.119	0.517±0.263	0.219-1.281	0.558±0.326
NO_2-N (mg/L)	ND-2.626	0.396±0.842	0.054-0.785	0.318±0.273
NO_3-N (mg/L)	2.532-19.663	12.229±6.495	2.372-22.249	13.357±7.695
COD_{Mn} (mg/L)	6.45-16.98	9.54±3.49	7.84-22.04	11.03±4.44

Table 4. Water quality measurements for the outdoor traditional ponds.

Pond size	Variable	Range	$\bar{x} \pm SD$	Variable	Range	$\bar{x} \pm SD$
A	temperature(℃)	18.5-30.6	26.18±3.40	NO_2-N(mg/L)	0.134-6.652	0.64±0.74
B		18.0-30.7	26.20±3.46		0.025-3.556	0.13±0.44
A	SD (cm)	10-90	27.31±13.58	NO_3-N(mg/L)	0.100-8.293	1.26±1.20
B		15-65	26.23±12.00		0.056-8.032	0.89±1.18
A	SS (mg/L)	4-208	53.80±31.71	TAN (mg/L)	0.049-3.390	0.82±0.71
B		6-156	59.98±32.05		0.098-1.588	0.43±0.26
A	pH	7.32-9.60	8.05±0.50	TN (mg/L)	1.023-6.493	2.81±1.12
B		7.31-10.11	8.34±0.43		0.111-7.471	2.63±1.90
A	DO (mg/L)	2.23-14.76	5.79±2.10	TP (mg/L)	0.030-0.849	0.37±0.14
B		3.83-15.26	7.85±2.15		0.068-1.731	0.40±0.32
A	COD_{Mn} (mg/L)	6.42-31.21	15.19±5.50	PO_4-P (mg/L)	0.004-0.391	0.09±0.08
B		7.17-44.41	17.62±9.48		0.004-0.242	0.04±0.04
A	Chl.a (mg/L)	0.003-0.29	0.10±0.07			
B		0.003-0.511	0.16±0.13			

Table 5. Water quality measurements for the outdoor eco-culture ponds.

Variable	2010 (pond number=3)		2011 (pond number=17)	
	Range	$\bar{x} \pm s$	Range	$\bar{x} \pm s$
DO(mg/L)	3.20-11.70	5.23±1.68	3.60-12.10	5.58 ±1.98
temperature(℃)	18.8-30.7	25.6±4.32	26.00-31.70	29.07 ±1.51
pH	7.84-8.63	8.23±0.37	7.60-9.80	8.38± 0.46
COD_{Mn} (mg/L)	10.88-15.67	13.27±3.12	7.88-40.18	18.36± 6.98
Chl.a (mg/L)	0.03-0.81	0.23±0.11	ND-0.60	0.12 ±0.11
TAN (mg/L)	0.04-0.70	0.30±0.26	0.13-4.08	0.55 ±0.62
NO_2-N (mg/L)	0.02-0.461	0.13±0.08	ND-0.20	0.01 ±0.03
NO_3-N (mg/L)	1.01-2.67	1.64±0.57	ND-0.70	0.25 ±0.11
TN (mg/L)	0.43-5.15	2.57±1.56	0.58-6.79	2.41 ±1.31
PO_4-P (mg/L)	0.006-0.24	0.13±0.06	ND-0.70	0.05 ±0.08
TP (mg/L)	0.04-0.371	0.18±0.14	0.03-0.80	0.22 ±0.13

The amount of culturable heterotrophic bacteria in traditional ponds water was higher than that in eco-culture ponds. And no *vibrio* was detected in eco-culture ponds while *vibrio* was detected in 52% water samples from traditional ponds even if there showed no obvious disease outbreak. The three kinds of specific virus including WWSV, TSV and IHHNV were not detected in shrimps of the two different types of ponds (Table 6). It seemed that good water quality in eco-culture ponds would affect the amount of bacteria thus lower the probability of bacterial infection.

Table.6 Monitoring results of culturable heterotrophic bacteria and vibrio in water and virus analysis results in two types of culture ponds in 2010.

Pond type	culturable heterotrophic bacteria (cfu/mL)	Vibrio (cfu/mL)	WWSV	TSV	IHHNV
Traditional ponds (n=22)	1.3×10^2-1.9×10^6	ND -1.4×10^4	ND	ND	ND
Eco-culture ponds (n=3)	$1.4\times10^2\pm6.7\times10^4$	ND	ND	ND	ND

ND: not detected

In 2010, shrimp production per crop in traditional ponds was lower than in indoor RAS or eco-culture ponds (Table 7). Good water quality seemed valuable in improving shrimp production. But in 2011, there was a disease outbreak in ponds with shrimp from Hainan, which spread to the other ponds. Virus monitoring showed that WWSV were found in infected shrimp. By September 26, there were only two ponds with shrimp that were still healthy, so production values could not be obtained but were obviously likely to be quite low. Water quality appeared to be quite good even when disease occurred (Table 5). SPF shrimp and disease prevention should be given a higher priority in these grow-out systems.

Table 7. Shrimp production in different experimented ponds in 2010.

Pond type	Production per crop (kg/m^2)
Indoor RAS	0.80-1.40
Traditional ponds	0.29-0.44 (Pond A) , 0.20-0.23 (Pond B)
Eco-culture ponds	0.80-0.92

The nutrient budget was analyzed in indoor recirculating systems in 2010 (Figure 4). Feed input 94.95% of total nitrogen and 97.06% of phosphorus, with only 4.75% of nitrogen and 2.93% of phosphorus originating from water. Since shrimp juveniles were so small, they accounted for only 0.30% and 0.01% of the input. After 100 days of culture, 34.24% of input was bound shrimp tissue, 53.84% was in the water, and 0.825% was in organisms attached to purifying nets. Shrimp retained only 16.84% of the phosphorus while the unaccounted proportion was quite high at 34.53%.

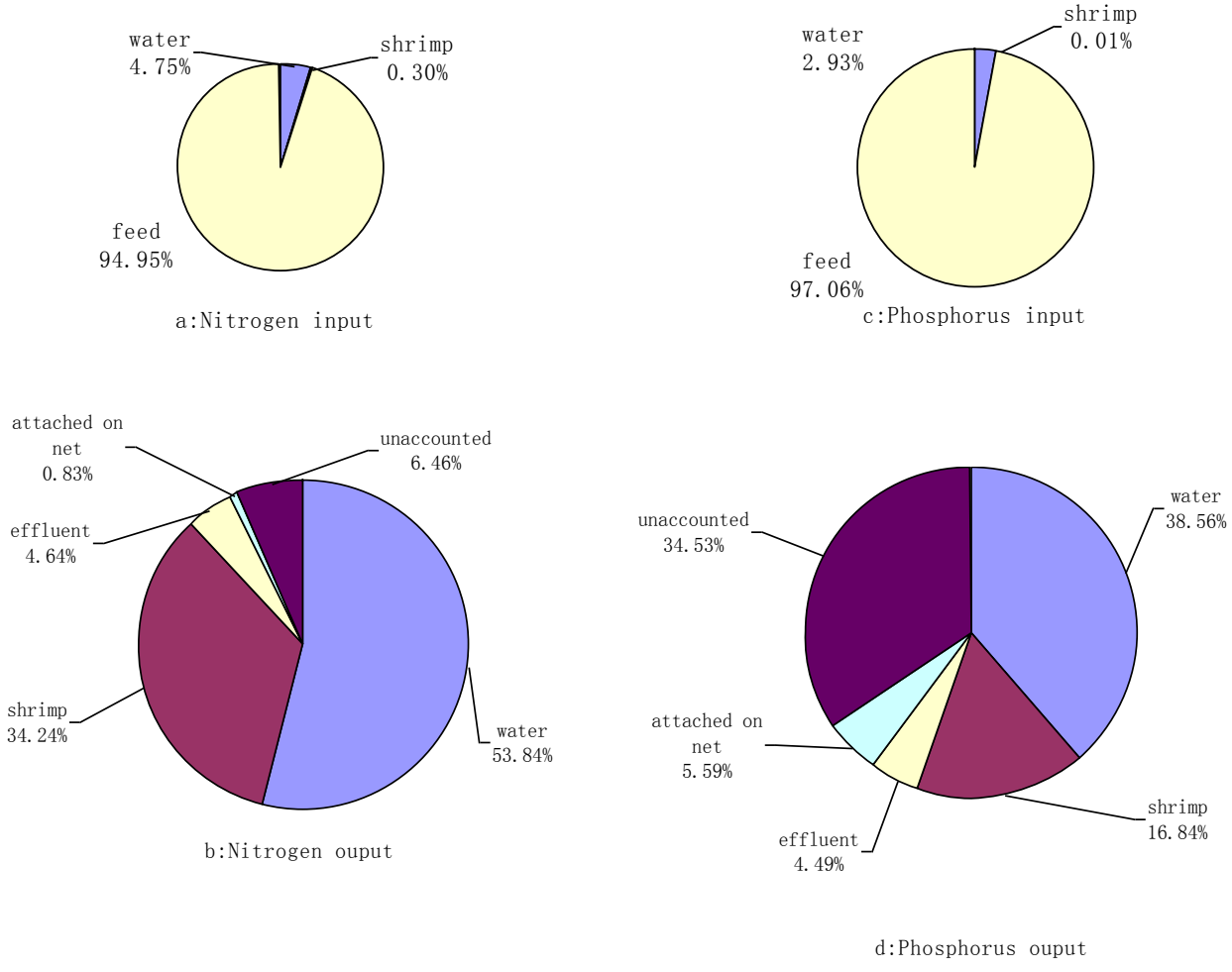


Figure 4. Nutrient budget for the indoor recirculating system.

Feed nutrient input was higher than optimum (Dhirendra et al., 2003). In Dhirendra's research over 90 days in closed systems, shrimp feed accounted for 76-92% of input nitrogen and 70-91% of phosphorus, while major sinks of nutrients were in the sediment (14-53% nitrogen and 12-29% phosphorus). In that study, the drained water at harvest contained 14-28% of input nitrogen and 12-29% of phosphorus.

Li et al. (2007) found that an intensive *Litopenaeus vannamei* culture had 84.3%-98.3% of nitrogen and 93.2%-97.3% of phosphorus inputs from feed. The major outputs of nitrogen and phosphorus were sediment (30.9%-43.9% and 51.5%-60.7%) and water exchange (27.5%-36.3% and 8.4%-23.9%). Their nutrient budget showed that only 14.5%-28.7% of nitrogen and 7.4% and 16.5% of phosphorus were transformed into harvested shrimp, which was a much lower retention rate than that in our RAS system.

Nitrogen and phosphorus inputs showed similar patterns in both traditional ponds and eco-culture ponds (Table 8). Nitrogen input to ponds was mostly from feed (85.21% and 88.06%), then water (14.68% and 11.85%). Nitrogen retention in the two types of ponds was different. Since aquatic plants were grown in eco-culture ponds, 7.89% of nitrogen was incorporated into plants, and the percent of nitrogen retained in water and sediment was less than that in traditional ponds. Phosphorus input also came mostly from feed (92.34% and 94.89%) and phosphorus retention was mainly in sediment (48.78% and 38.12%).

Table 8. Nutrient budgets for traditional ponds and eco-culture ponds.

		Traditional ponds	Eco-culture ponds
Nitrogen input (%)	Feed	85.21	88.06
	Water	14.68	11.85
	Shrimp	0.11	0.09
Nitrogen output (%)	Shrimp	31.02	35.73
	Water	19.45	11.32
	Sediment	33.24	27.09
	Aquatic plant	-	7.89
	Unaccounted	16.29	17.97
Phosphorus input (%)	Feed	92.34	94.89
	Water	7.57	5.04
	Shrimp	0.09	0.07
Phosphorus output (%)	Shrimp	13.21	15.25
	Water	11.36	5.69
	Sediment	46.78	38.12
	Aquatic plant	-	6.22
	Unaccounted	28.65	34.72

In 2010, algae blooms broke out in two traditional ponds, so we tried to determine the relationship between chlorophyll-a and other water quality parameters. Chlorophyll-a had highly significant positive correlations to SS, COD_{Mn}, TN and TP. A significant positive correlation also existed between chlorophyll-a and DO. Highly significant negative correlations were found between chlorophyll-a and SD

and between chlorophyll-a and PO_4-P. No significant correlations were found between chlorophyll-a and water temperature, pH, NO_2^--N, NO_3^--N and NH_3-N. According to the methods for selecting independent variables in multiple linear regression analysis, four water quality parameters including COD_{Mn}, TN, PO_4-P, and TP were used to determine the stepwise regression model which was

$$\text{Chl.a} = -0.03457 + 0.0217\,\text{TN} + 0.0007\,\text{COD}_{\text{Mn}} - 0.49\,\text{PO}_4\text{-P} + 0.338\,\text{TP},\ (r = 0.6896)$$

The effects of these four factors to chlorophyll-a were tested using partial regression coefficients. The most influential water quality parameter to chlorophyll-a was TN, and then TP, PO_4-P, and COD_{Mn} in turn.

Using the multiple regression formula, we calculated chlorophyll-a and compared it with measured values (Figure 5). The results matched reasonably well, especially when the concentration of chlorophyll-a was high.

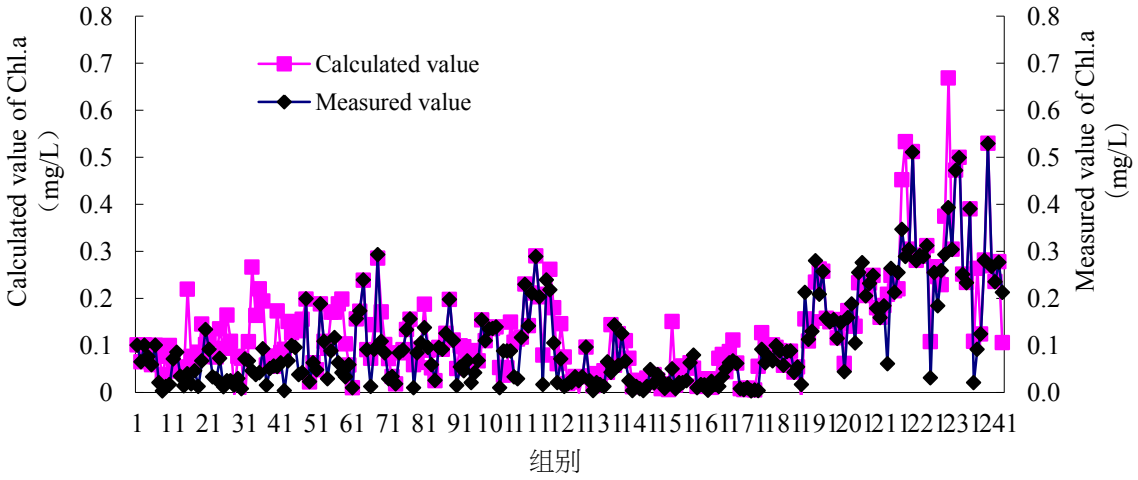


Figure 5. Comparison between calculated and measured chlorophyll-a in traditional ponds in 2010.

We did same statistical analysis on data from 17 eco-culture ponds in 2011 and following equation was obtained:

$$\text{Chl.a} = 0.21809 + 0.00590\text{COD}_{\text{Mn}} - 0{,}0092\text{t} + 0.340\text{TP} - 0.32\text{PO}_4 - \text{P}\ \ (\text{r} = 0.7032)$$

Compared to traditional ponds, TN was not included in the regression for eco-culture ponds. Combined technologies of purifying nets and aquatic plants probably played important roles in reducing nitrogen content of the water, resulting in low correlation between TN and chlorophyll-a. Phosphorus had a closer relationship with chlorophyll-a, so it is essential to keep phosphorus content low in water. According to the budget, over 90% of P came from feed but less than 16% of P was converted to shrimp. Improving utilization rate of feed P is important not only in cost savings, but also in controlling pond algae blooms.

ANTICIPATED BENEFITS

Recently, farmers have been encouraged by local government to culture shrimp or other aquatic animals with standardized methods, which mean acceptable pond types, preferred feed, SPF shrimp, suggested water quality control technologies, and permitted chemical use. But aquaculture is a complicated industry and successful production depends on not only technology, but also on management experience, shrimp quality, and even weather. In this study, we compared three types of shrimp culture models, analyzed water quality and production differences, evaluated the nutrient budgets and relationship between chlorophyll-a and other parameters. Combined water quality control technologies can be extended to other farmers. Some farmers have already begun to use purifying nets, bottom aeration in their ponds, and aquatic plants or aquatic vegetables to remove nutrients.

In short-term training to farmers, we taught them how to monitor water quality and showed them the basic technology for eco-culture of shrimp. Further research is still needed, particularly related to disease control.

LITERATURE CITED

Chang-fa, L., 2002. Environmental friendship aquaculture – Zero discharge integrated recirculating aquaculture systems. Journal of Dalian Fisheries University, 17:220-226.

Chen H.C., 1998. Studies on the better management for highly successful intensive shrimp culture.5th Asian Fish Forum, Thailand, 13 p.

Dhirendra, P.T., and C.K. Lin, 2003. Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. Aquacultural Engineering, 27:159-176.

Li, Y., L. Jian, and W. Qingyin, 2007. Effect of stocking density on input and output of nitrogen and phosphorus in super-intensive shrimp farming pond. Journal of Fishery Sciences of China, 14:926-931.

Sung-Koo, K., I. Kong, B.H. Lee, L. Kang, M.G. Lee, K.H. Suh, 2000. Removal of ammonium-N from a recirculation aquacultural system using an immobilized nitrifier. Aquacultural Engineering, 21:139-150.

Ying, L. 2001. Control of pH and design of nitrification facilities in closed cycle aquaculture systems. Fishery Modernization, 3:10-11, 35.

Part 2. Objective 4
Effects of *Microcystis Aeruginosa* on Juvenile Survivial and on Enzyme Activites of Adult Crayfish (*Procambarus Clarkii)*

Liu Liping, Yue Yaling, Li Kang, Chen Taoying, and Jiang Min
Shanghai Ocean University
Shanghai, China

James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

ABSTRACT

In this paper, larvae of the crayfish *Procambarus clarkii* were exposed to different concentrations of the algae *M. aeruginosa* (1.0×10^6, 5.0×10^6, 1.0×10^7, and 2.0×10^7 cells/mL) to investigate the algae's impact on larvae survival and hepatopancreatic ultrastructure. At the same time, adult crayfish were exposed to the same algae concentrations and the total hemocyte count (THC), serum hemocyanin content, and superoxide dismutase (SOD), peroxidase (POD), phenoloxidase (PO), and Na^+/K^+-ATPase activities in gill filaments were evaluated. The results showed that: (1) *M. aeruginosa* significantly reduced larval survival rate, such that, when exposed to 1.0×10^7 cells/mL algae, the survival rate was significantly lower than controls by day 19 ($p<0.05$) and, at the end of the 30 d exposure, survival rate was 48%. (2) Microscopic observations showed that the larval hepatopancreas became darkened and, under transmission electron microscopy, the tissue's cells were observed to be damaged. (3) At the highest algae density, the crayfish exhibited a stress reaction in which their THCs increased significantly and stayed high after being exposed for 1 d. There were no significant differences in hemocyanin content at the beginning of algae exposure, but it was significantly decreased by 5 d ($p<0.05$). Serum SOD activity was inhibited after 1 d of algae exposure and appeared elevated to a higher level by 5 d. POD and PO activities showed fluctuating trends, and Na^+/K^+-ATPase activity in gill filaments dropped significantly after 1 d ($p<0.01$), then increased, and finally remained at a higher level. These results indicated that *M. aeruginosa* exerted a strong negative impact on juvenile crayfish survival and affected the immunity status of adult crayfish, which may cause decreased adult growth from the elevated stress.

INTRODUCTION

Procambarus clarkii is a crayfish that exists in fresh water, such as channels and ponds, and is widely cultivated, particularly in Hubei and Jiangsu provinces, because of their delicious taste and tolerance for poor environmental conditions. However, cyanobacteria blooms occur frequently due to water eutrophication (Zha et al., 2004, 2007; Zhang et al., 2001), which has drawn academic attention to the blooms and their potentially harmful metabolic products. Research in the past ten years has shown that *Microcystis aeruginosa* in natural water and its toxin microcystin have toxicological effects (Reimkainen et al., 2001; Andersen et al., 1993; Zurawell et al., 2004; Orr et al., 2001; Park et al., 2001; Zimba et al., 2006) on organisms, and cyanobacteria blooms can alter water quality, while the toxin itself can accumulate in aquatic organisms and move up the food chain, causing severe damage to aquaculture and human health. However, the impact of *M. aeruginosa* on *P. clarkii* has received little study and been rarely reported.

In this paper, larvae and adult of crayfish *P. clarkii* were exposed to different *M. aeruginosa* concentrations to investigate the impacts of the algae on larval survival as well as total serum hemocyte density (THCs), serum hemocyanin content, superoxide dismutase (SOD), peroxidase (POD), phenoloxidase (PO), and Na^+/K^+-ATPase activities in gill filaments of adults. Moreover, the effect of *M.*

aeruginosa on larval body color and hepatopancreas ultrastructure were examined by visual and transmission electron microscopy (TEM), with the goal to observe the chronic toxicological mechanism of this algae on crayfish and to provide some useful information to aid healthy cultivation of crayfish.

MATERIAL AND METHODS

The larvae of *P. clarkii* (body length: 12.09 ±0.98 mm, body weight: 99.20 ±13.80 mg) were contributed by the Aquatic Life Vegetative Reproduction Laboratory of Shanghai Ocean University, and adult crayfish (body length: 57.52 ±3.84 mm, body weight: 16.90 ±2.10 g) from Shanghai Mudflat Resource Development Institute. Experimental crayfish were raised in fresh water after it was sterilized with 5% salt solution and full aeration, after which, healthy individuals of similar size were selected randomly for experimentation. *M. aeruginosa* was contributed by the Institute of Hydrobiology, Chinese Academy of Sciences, and maintained in culture solution BG-11 for 15 d, at 20 ±1℃, with a 12/12 light/dark cycle.

In accordance with the usual algae density in *M. aeruginosa* blooms (Lu et al., 2005; Vasconcelos et al., 2001) three concentrations were selected: 1.0×10^6, 5.0×10^6, and 1.0×10^7cells/mL, to examine the effect of *M. aeruginosa* on juvenile *P. clarkii* survival. A control group was maintained in fresh water previously sterilized by boiling. All treatments were triplicated. The experimental vessel was a square plastic 5 L casing with 4 L of water. Healthy crayfish larvae were divided randomly into groups of 20 per casing, fed daily at a fixed time, and the water renewed every two d to maintain a steady algae density. The surviving larvae were counted daily with death based on lack of appendage movement.

Healthy *P. clarkii* larvae were cultivated with *M. aeruginosa* at 5.0×10^6, 1.0×10^7, and 2.0×10^7 cells/mL for 7 d and their hepatopancreas removed after the larvae were washed in sterile water. The collected tissue was fixed in 2.5% glutaraldehyde and 1% osmic acid, and dehydrated in ethanol. Next, tissue was embedded in epoxy resin EPON812, microtomed into ultrathin sections with a LKB NOVA slicing machine (LKB Nova, Bromma, Sweden), dyed twice with uranyl acetate and lead citrate, and observed by transmitting electron microscope (TEM, H-700, Hitachi, Ltd., Japan) with an accelerating voltage of 75 kV.

Triplicate groups of 60 randomly selected healthy *P. clarkii* adults were cultivated with *M. aeruginosa* at 1.0×10^6, 5.0×10^6, 1.0×10^7 cells/mL, a control group in sterilized fresh water. Each group was cultivated in a 200 L plastic storage box and evaluated for THC and serum hemocyanin content, as well as SOD, POD, PO, and Na+/K+-ATPase activities of their gill filaments. THC was assessed using the method of Yao et al. (2007), PO activity with the method of Ashida (1971), and hemocyanin content using the combined methods of Johnson et al. (1984) and Zhang et al. (2005). Also, in consideration of the strong hemocyanin absorption peak at 280 and 334 nm, the spectrophotometer was adjusted to measure optical density at both OD_{280} and OD_{334}. An examination reagent box from Nanjing Jiancheng Company was used to measure the SOD, POD, and Na+/K+-ATPase.

RESULTS

Juvenile crayfish survival was significantly correlated with the *M. aeruginosa* concentration. There was no reduction in survival when algae density was 1.0×10^6 cells/mL; young crayfish began to die after 25 d of exposure, and at the end of cultivation (30 d), survival rate was 95%, with no significant difference from the control. However, when algae density was 5.0×10^6 cells/mL, survival rate at 26 d was only 68.33%, significantly lower than the control ($p<0.05$). In addition, when algae density reached 1.0×10^7 cells/mL, survival was only 61.67% after 19 d of exposure, also significantly lower than the control ($p<0.05$), and at the end of this exposure, survival rate was only 48.33% (Figure 1).

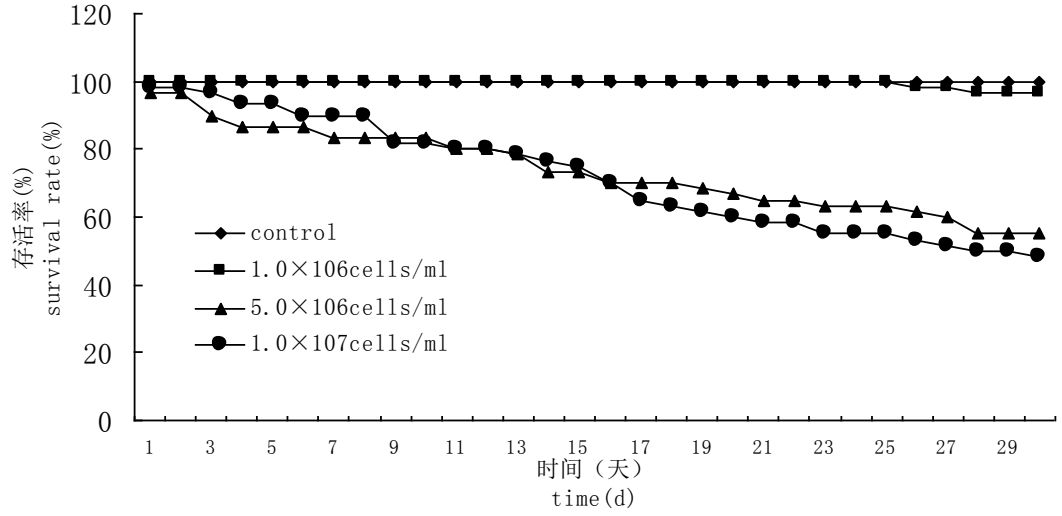


Figure 1. Effect of different densities of *M. aeruginosa* on survival rate of *P. clarkii* larvae.

Observation by anatomical lens revealed that, compared to controls, *P. clarkii* larvae showed differences in testa spots and body color when cultivated in different densities of *M. aeruginosa*. Specifically, testa spots and body color were darker in *M. aeruginosa* at density of 1.0×10^7 and 2.0×10^7 cells/mL. Closer observation of the carapace revealed that the hepatopancreas was swollen to varying degrees (Figure 2).

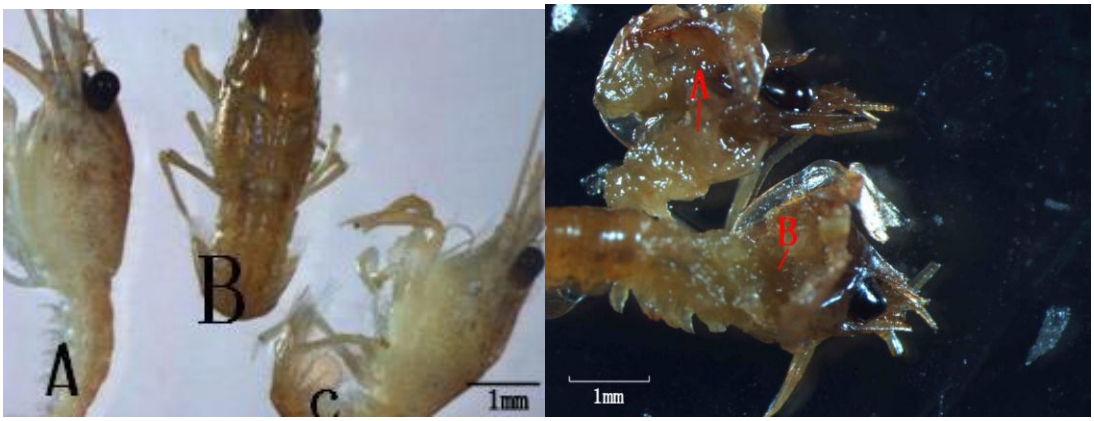


Figure 2. Effect of *M. aeruginosa* on body color of crayfish larvae. Left, morphology of whole body; right, hepatopancreas; A, 5.0×10^6 cells/mL; B, 1.0×10^7 cells/mL; and C, control.

TEM images of larval hepatopancreas revealed damaged hepatopancreatic cells in crayfish cultivated at 1.0×10^7 and 2.0×10^7 cells/mL algae (Figure 3), including damage to cell chromatin around the nuclear membranes, nucleolus margination, large cytoplasmic vacuoles, and organella damage; while hepatopancreatic cells of the control group were structurally complete, with evenly distributed chromatin, and lipid droplets similar to a yolk or fat granule. The group reared in the lowest algae density also had relatively complete cell membranes in their hepatopancreas, with no obvious damage.

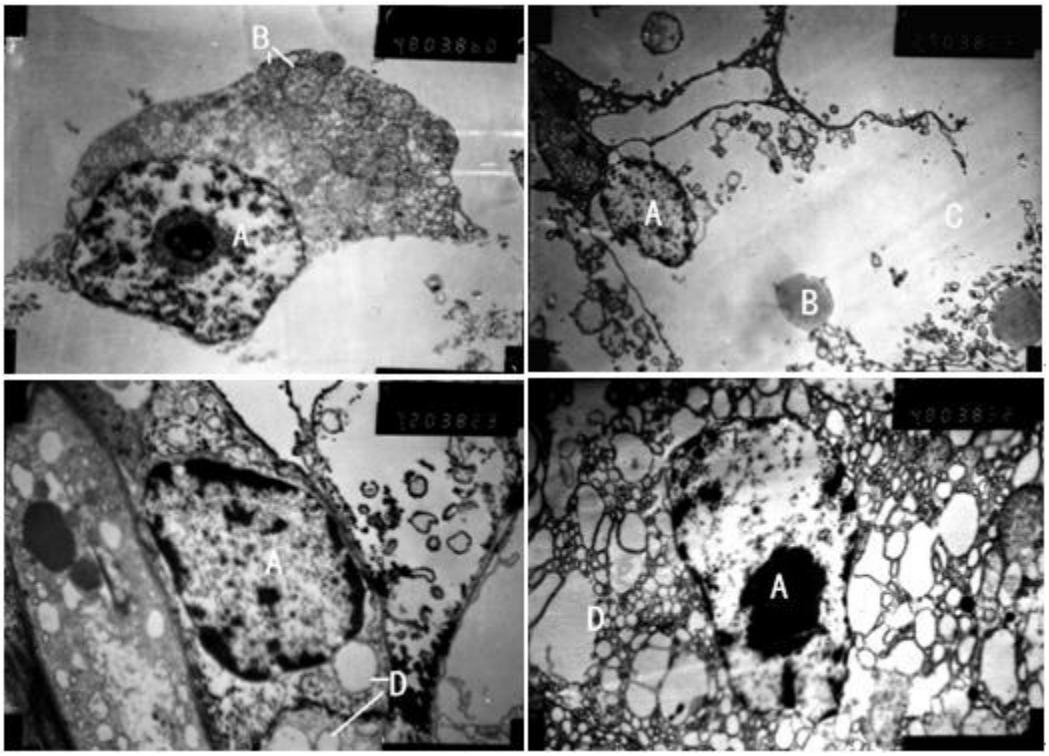


Figure 3. Ultrastructure of crayfish larvae hepatopancreas. A, cell nucleus; B, cellular organelle/lipid droplet; C, cell membrane breakage site; D, vacuoles between cytoplasm.

The THCs of adult *P. clarkii* were increasingly affected with increasing *M. aeruginosa* density (Figure 4); while the THCs of controls were basically the same as the 1.1×10^6 cells/mL treatment group. After exposure to algae-laden water for 24 h, adult crayfish THCs in each experimental group rose, with higher density groups at 5.0×10^6 and 1.0×10^7 cells/mL showing significant differences compared to the control ($p<0.01$). The lowest density group also rose significantly in THC after three days of exposure. After exposure to *M. aeruginosa*, the THC of all experimental groups was sustained at relatively high values.

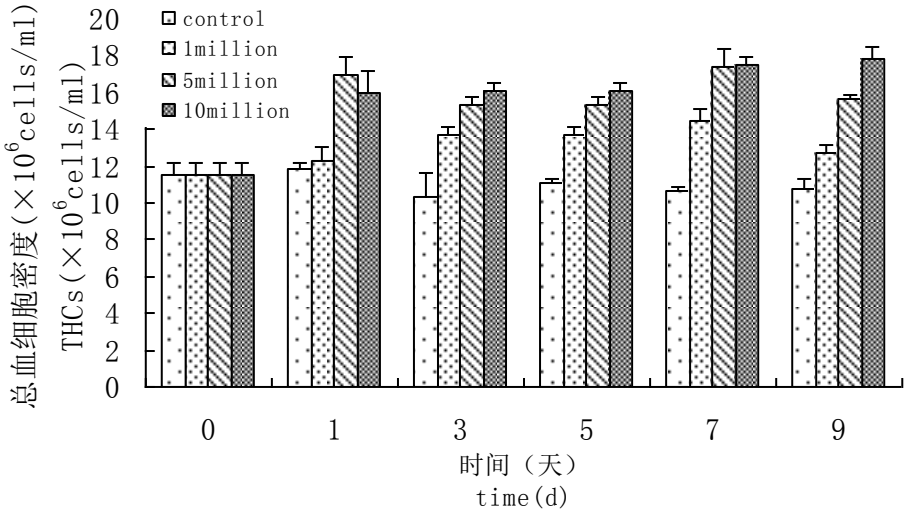


Figure 4. Effect of *M. aeruginosa* density on adult crayfish THCs.

Evaluation of the effect of *M. aeruginosa* density on *P. clarkii* hemocyanin revealed that the serum hemocyanin concentration experienced no significant variation after 3 d of algae exposure but decreased significantly after 5 d (Figure 5), with the hemocyanin concentration of the two higher algae density groups significantly lower than the control ($p<0.01$). Soon afterwards, hemocyanin returned to a concentration similar to controls in all groups. Moreover, the hemocyanin spectrophotometer measurements at 280 and 334 nm were similar.

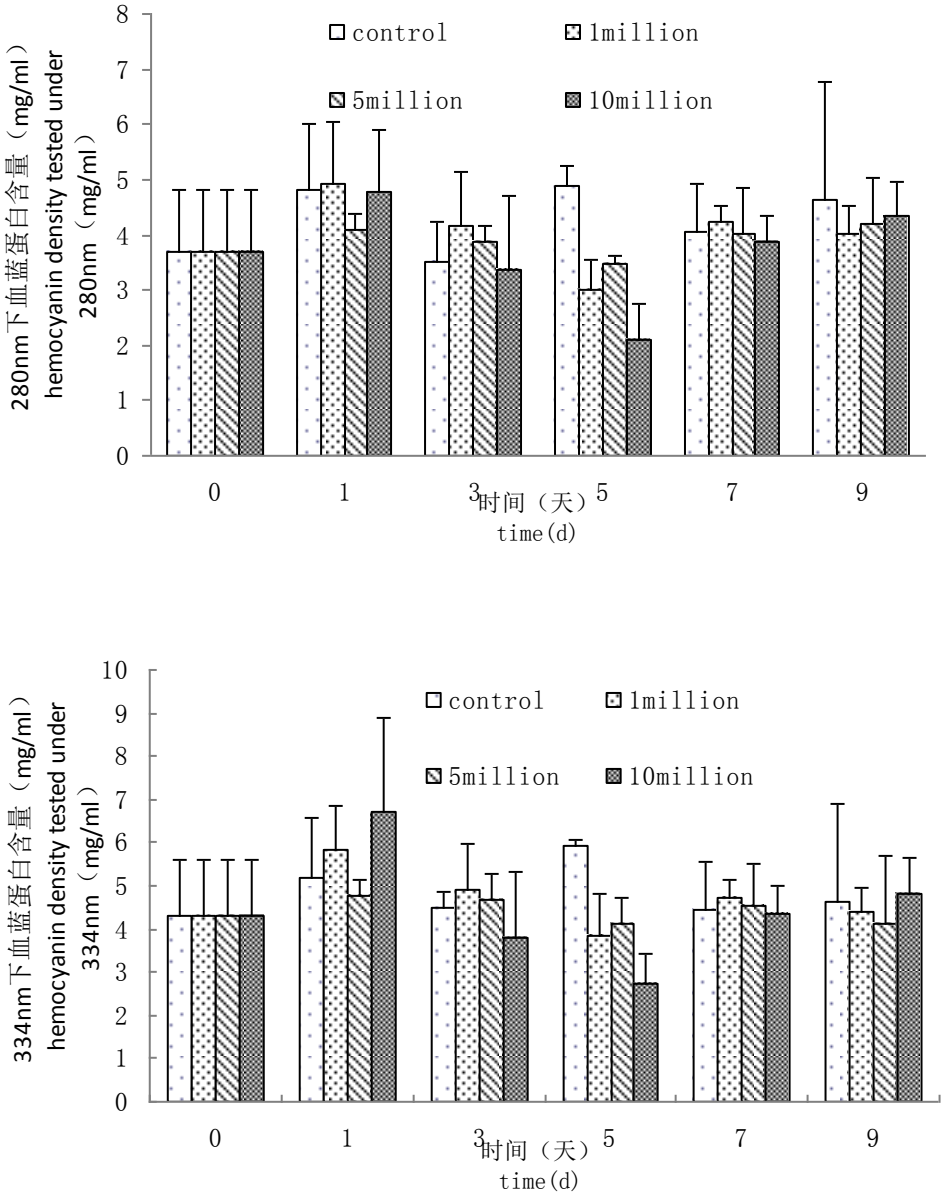


Figure 5. Effect of *M. aeruginosa* density on adult crayfish hemocyanin. Top, 280 nm; bottom, 334 nm.

Evaluation of the effect of *M. aeruginosa* density on adult *P. clarkii* PO activity revealed that after exposure to algae at 1.0×10^7 cells/mL for 1 d, PO activity in the serum increased significantly ($p<0.01$),

but soon afterwards, began to decrease and was finally restored to initial values (Figure 6). In addition, PO activity in groups with 1.0×10^6 and 5.0×10^6 cells/mL were not significantly different from the control.

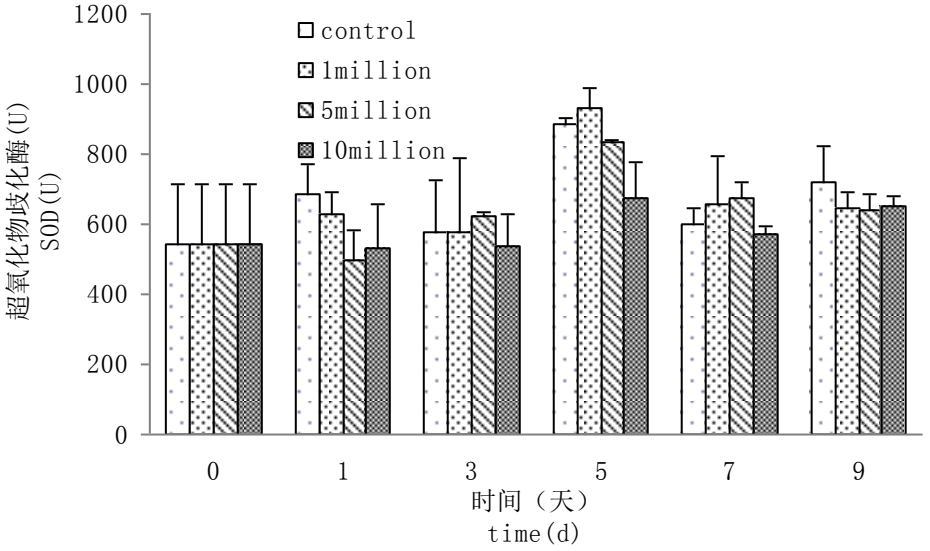


Figure 6. Effect of *M. aeruginosa* density on adult *P. clarkii* PO activity.

M. aeruginosa in lower concentrations, 1.0×10^6 and 5.0×10^6 cells/mL, produced no significant effect on *P. clarkii* SOD activity, but at the highest concentration, SOD activity was significantly restrained (Figure 7). SOD activity in the highest algae group decreased slightly after 1 d of exposure and dropped significantly after 5 d of exposure ($p<0.01$).

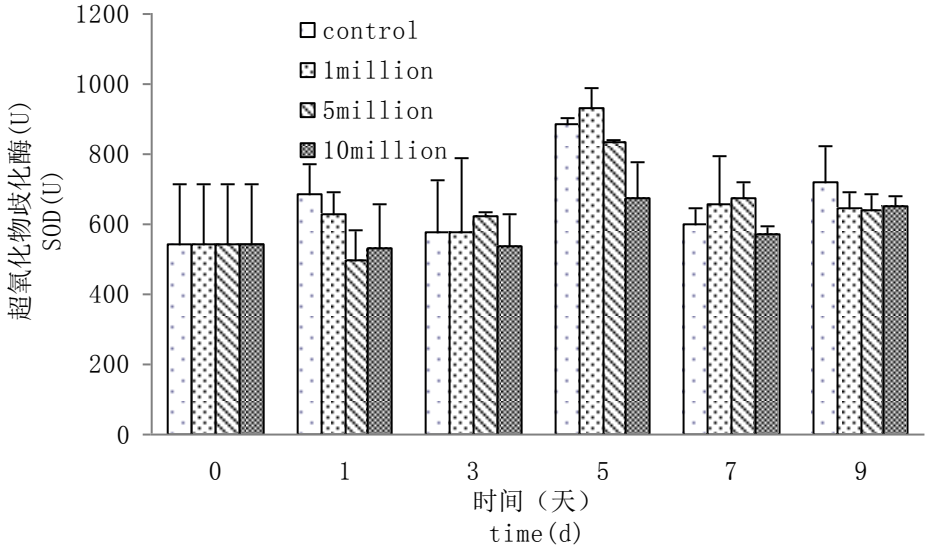


Figure 7. Effect of *M. aeruginosa* density on adult crayfish SOD activity.

In contrast, after exposure to *M. aeruginosa*, POD activity in adult crayfish demonstrated a tendency of rising and then falling. In the high algae group, POD activity rose significantly after 1 d of exposure

($p<0.05$), and in the two lower algae groups, POD activity increased slightly after 3 d and, soon afterwards, POD activity of all experimental groups returned to the level of the control (Figure 8).

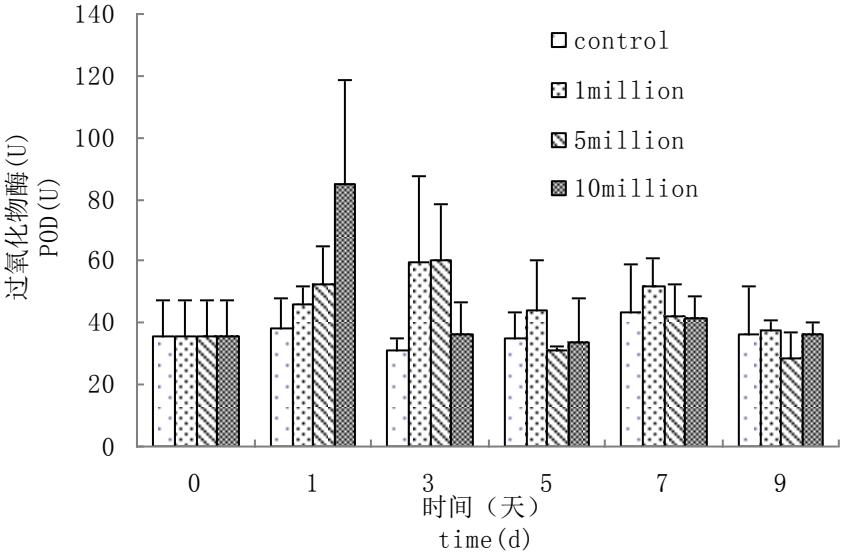


Figure 8. Effect of *M. aeruginosa* density on adult crayfish POD activity.

The effect of *M. aeruginosa* exposure on the serum Na^+/K^+-ATPase activity of adult *P. clarkii* showed a tendency of declining and then rising (Figure 9). Specifically, Na^+/K^+-ATPase activities in all 3 algae groups declined significantly after 1 d of exposure, returned to their initial values after 3 d, strengthened gradually until reaching a peak at 7 d, and then decreased again after 9 d of exposure.

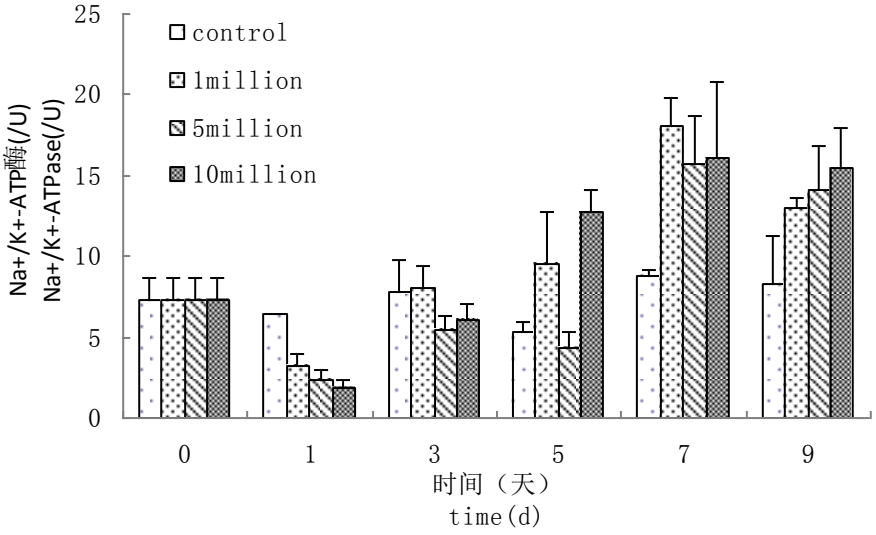


Figure 9. Effect of *M. aeruginosa* density on adult crayfish Na^+/K^+-ATPase activity.

DISCUSSION

Survival And Health Status Of *P. Clarkii* Larvae Were Influenced by *M. aeruginosa* in the culture water, which corresponded to the fact that cyanobacteria blooms in water bodies cause death in different organisms (Babica et al., 2006; Zimba et al., 2001; Xie et al., 2004; Obernum et al., 1999; Yokoyama and Park, 2002, 2003), and indicated that even crayfish with strong environmental tolerances were affected by *M. aeruginosa*. Microcystin is a type of hepatotoxin, which can result in liver swelling and congestion, increasing the liver to body weight ratio and causing a series of enzymological changes. Moreover, this toxin can induce apoptosis in various cells (Amorim and Vasconcelos, 1999; Fu et al., 2004; Lankoff, 2004) and cause framework damage to cell ultrastructures (Beasley et al., 2000). In this study, the larval hepatopancreas was found to experience swelling to different degrees and hepatopancreas necrocytosis after algal exposure for 7 d, but no apoptotic body or cell nucleus breakage was observed in the process. Therefore, the larval hepatopancreas was strongly affected by high concentration of microcystin, resulting in large quantities of necrocytosis. However, further research is required to understand whether *M. aeruginosa* at low density can also induce hepatopancreatic apoptosis.

Among crustaceans, hemocyte count and PO activity have direct correlations with immunity. Blood lymphocytes hold a central position in the immune system and THC can reflect, to some extent, the immunological stress, or physical condition of an organism and has been adopted as one of the indicators for measuring immunity level of crustaceans (Huang et al., 2007). *M. aeruginosa* caused THCs to rise in *P. clarkia* during our exposures, indicating that the algae stimulated their immune system to generate more blood lymphocytes in an effort to resist the adverse environment. PO is the most important antioxidizing element in crustacean body fluid and is the first defensive line against external damage, yielding PO activity as a common indicator of immunity level. PO activity strengthened significantly after algal exposure in this study, which was similar to results reported by Huang et al. (2007) and Lei et al. (2001) that PO activity strengthened with different infection status. As the respiratory pigment of crustaceans, hemocyanin is dispersed in the hemolymph, performing oxygen transport and participating in immune defense, exhibiting functions similar to phenoloxidase under the influence of certain substances (Adachi et al., 2003). Yoganandhan et al. (2003) found that the hemocyanin ratio in *Penaeus indicus* declined significantly after WSSV infection, which agrees with the results in our study, and this may indicate that the algae caused hemocyanin decomposition and reduced oxygen transport, and damage to the crayfish immune system. When algal density reached a higher level, it rapidly stimulated the crayfish immune system, generating large numbers of blood lymphocytes and releasing phenoloxidase as well as similar immunological factors to participate in the immune response.

Antioxidases, such as SOD and POD, are important in metabolic detoxification in organisms. SOD is a crucial enzyme in balancing oxidation and antioxidation, and variation in its activity directly reflects the ability to eliminate free radicals in the organism itself. Research has demonstrated that SOD activity declines significantly when aquatic organisms are in an adverse environment (Hu et al., 2009; Zhang et al., 2007).

In this study, the SOD activity of *P. clarkii* was also found to decline significantly after exposure to *M. aeruginosa*, which was similar to a report by Sun and Ding (1999) on the effect of ammonia nitrogen on the premonition of *Penaeus chinesis*. POD performs the function of catalytic decomposition of oxides or peroxides, as well as oxygenolysis of toxins (Ashida and Soderhall, 1984; Soderhall and Cerenius, 1998). In the current work, serum POD activity of crayfish rose significantly after exposure to high-density algae for 1 d, demonstrating that oxides increased significantly in the crayfish with algae exposure and that POD was generated in large amounts by the organisms and could have helped eliminate excessive internal oxides. The changes of antioxidase activity observed here may have been caused by the *M. aeruginosa* generated microcystin MC-LR, which produced damage after crossing the cell membrane. Research has indicated that, after entering cells, algae toxins can produce oxidative damage, lipid peroxidation, DNA chain breakage, and oxidative modification of proteins (Ding et al., 2000; Chen et al., 2006; Fan et al., 2008), which are features that appear common to the toxicological mechanisms of *M. aeruginosa*.

For crustaceans, Na^+/K^+-ATPase is an important osmoregulatory enzyme, mainly scattered in the gill, hepatopancreas, and antennal gland, and its activity is an important measure of an organism's physiological metabolism and growth status (Pan and Liu, 2005). In the present study, Na^+/K^+-ATPase activity changes observed in gill filaments were similar to results of Zhao et al. (2006) on the effect of salt concentration on Na^+/K^+-ATPase activity in *Acipenser schrenckii* gill tissue. One possible explanation for this effect is that gill tissue had direct access to the algae in the external environment and experienced a stress response when Na^+/K^+-ATPase activity declined rapidly. However, after residing in an adverse environment for some time, the crayfish entered into an active adjustment stage in which Na^+/K^+-ATPase activity increased significantly, regulating the cell osmotic pressure and performing the functions of respiration, excretion, osmoregulation, and disease defense. Consequently, the crayfish successfully adapted to the external environment.

The stress response in organisms is an active fight against adverse environmental conditions and when abrupt changes in environmental factors exceed the crustaceans' endurance, metabolic disorders, osmoregulation dysfunction, immunity decline, or even death can result (Guo et al., 2007). When the external environmental factors are within their endurance levels, they can adapt with a buffering stress response. The experimental results here confirmed that: the significant death rate in juvenile *P. clarkii* was caused only when the *M. aeruginosa* density reached 1.0×10^7 cells/mL, beyond the endurance of the juveniles. However, the adult enzyme activities experienced increases and decreases at a variety of exposure densities, finally stabilizing and demonstrating that when the algae density reached 1.0×10^7 cells/mL, adults managed to show great tolerance, gradually adapting to the external environment after a clear stress response stage.

ACKNOWLEDGMENTS

The authors of this paper want to express their gratitude to General Manager Shen Hong from Shanghai Mudflat Resource Development Institute who provided generous support for this research, and also to Dr. Wang Chun and undergraduate students He Yue-Feng and Wang Jia-Qi in Shanghai Ocean University, who have given their kind help in the experiments.

LITERATURE CITED

Adachi, K., T. Hirata, and T. Nishioka, 2003. Hemocyte components in crustaceans convert hemocyanin into a phenoloxidase-like enzyme. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 134:135-141.

Amorim, A., and V. Vasconcelos, 1999. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. Toxicon, 37:1041-1052.

Andersen, R.J., H.A. Luu, and D.X.Z. Chen, 1993. Chemical and biological evidence links microcystins to salmon "netpen liver disease." Toxicon, 31:1315-1323.

Ashida, M., 1971. Purification and characterization of pre-phenoloxidase from hemolymph of the silkworm *Bombyx mori*. Archives of Biochemistry and Biophysics, 144:749-762.

Ashida, M. and K. Söderhäll, 1984. The prophenoloxidase activating system in crayfish. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 77:21-26.

Babica, P., L. Bláha, and B. Maršálek, 2006. Exploring the natural role of microcystins-a review of effects on photoautotrophic organisms. Journal of Phycology, 42:9-20.

Beasley, V.R., R.A. Lovell, and K.R. Holmes, 2000. Microcystin-LR decreases hepatic and renal perfusion, and causes circulatory shock, severe hypoglycemia, and terminal hyperkalemia in intravascularly dosed swine. Journal of Toxicology and Environmental Health Part A, 61(4):281-303.

Chen, G., Z. Yang, and M. Tao, 2006. Toxicological study of fresh water microcystins. Journal of Hygiene Research, 35:120-122.

Ding, W., H. Shen, and C. Ong, 2000. The critical role of ROS and mitochondrial membrane permeability in microcystin-LR induced rapid apoptosis in primary rat hepatocytes. Hepatology, 32:547-555.

Fan, Y., S. Zhan, and J. Gan, 2008. Toxicity of microcystin and their prevention. Chemistry of Life, 28:101-103.

Fu, W.Y., M.W. Li, J.P. Chen, and L.H. Xu, 2004. Detection of apoptosis of renal cells of mice induced by microcystin-LR . Acta Hydrobiologica Sinica, 28:101-102.

Guo, C., Y. Guan, and B. Liu, 2007. Effect of temperature, pH and salinity on gill Na^+/K^+-ATPase activity in *Procambarus clarkii*. Chinese Journal of Zoology, 42:96-102.

Hu, G.F., Z. Li, H.W. Liang, C.Z. Wang, Q.C. Wu, X.Z. Luo, and G.W. Zou, 2009. Effects of Cadmium on SOD and CAT in hepatopancreas, antennary gland and gill of *Procambarus clarkii*. Journal of Agro-Environment Science, 28:1806-1811.

Huang, X.X., H.Q. Zhou, and L.P. Song, 2007. The effect of acute infection on the innate immune activities of the shrimp, *Fenneropenaeus chinensis*. Acta Hydrobiologica Sinica, 31:325-331.

Johnson, B.A., C. Bonaventura, and J. Bonaventura, 1984. Allosteric modulation of *Callinectes sapidus* hemocyanin by binding of L-lactate. Biochemistry, 23:872-878.

Lankoff, A., 2004. The uptake kinetics and immunotoxic effects of microcystin-LR in human and chicken peripheral blood lymphocytes in vitro. Toxicology, 204:23-40.

Lei, Z.H., J. Huang, B. Yang, and K.K. Yu, 2001. Immune factors in haemolymph supernatant of *Penaeus chinensis* infected by WSSV. Journal of Fishery Sciences of China, 8(4): 46-51.

Lu, K.H., C.H. Jin, and Y.C. Wang, 2005. Control of cyanobacterial blooms in eutrophication lakes by tilapia. Journal of Fisheries of China, 29:811-818.

Oberemm, A., J. Becker, and G.A. Codd, 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. Environmental Toxicology, 14:77-88.

Orr, P.T., G.J. Jones, and R.A. Hunter, 2001. Ingestion of toxic *Microcystis aeruginosa* by dairy cattle and the implications for microcystin contamination of milk. Toxicon, 39:1847-1854.

Pan, L., and H. Liu, 2005. Review on the osmoregulation of crustaceans. Journal of Fisheries of China, 29:109-115.

Park, H.D., Y. Sasaki, and T. Maruyama, 2001. Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environmental Toxicology, 16(4):337-343.

Reinikainen, M., J.A.O. Meriluoto, and L. Spoof, 2001. The toxicities of a polyunsaturated fatty acid and a microcystin to *Daphnia magna*. Environmental toxicology, 16:444-448.

Söderhäll, K., and L. Cerenius, 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. Current Opinion in Immunology, 10:23-28.

Sun, J.J., and M.L. Ding, 1999. Effect of ammonia-N on anti-disease ability of *Penaeus chinensis*, Oceanologia Et Limnologia Sinica, 30:267-272.

Vasconcelos, V., S. Oliveira, and F.O. Teles, 2001. Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. Toxicon, 39:1461-1470.

Xie, L., P. Xie, and K. Ozawa, 2004. Dynamics of microcystins-LR and-RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. Environmental Pollution, 127:431-439.

Yao, C.L., C.G. Wu, and J.H. Xiang, 2007. Changes in antimicrobial activity of plasma and haemocytes in *Fenneropenaeus chinese* challenged by killed *Vibrio anguilarum* . Oceanologia Et Limnologia Sinica, 38(1): 1-7.

Yoganandhan, K., S. Thirupathi, and A.S. Hameed, 2003. Biochemical, physiological and hematological changes in white spot syndrome virus-infected shrimp, *Penaeus indicus*. Aquaculture, 221:1-11.

Yokoyama, A., and H.D. Park, 2002. Mechanism and prediction for contamination of freshwater bivalves(Unionidae) with the cyanobacterial toxin microcystin in hypereutrophic Lake Suwa, Japan. Environmental Toxicology, 17:424-433.

Yokoyama, A., and H.D. Park, 2003. Depuration kinetics and persistence of the cyanobacterial toxin microcystin-LR in the freshwater bivalve *Unio douglasiae*. Environmental Toxicology, 18:61-67.

Zha, G.C., C.Q, Zhou, J.R. Huang, J.G. He, and X.W. Mai, 2004. Studies on the structure and biodiversity of the microplankton community in *Litopenaeus vannamei* desalination culture ponds. Acta Ecologica Sinica, 24(8):1752-1759.

Zha, G.C., C.Q. Zhou, X.G. Niu, 2007. Harm of *Microcystis aeruginosa* to *Litopenaeus vannamei* Low Salinity Stocking. Acta Scientiarum Naturalium Universitatis Sunyatseni, 46(2):64-67.

Zhang, J., J.X. Wang, Y.J. Zhang, C.Q. Liu, and H.T. Cui, 2007. Effect of starvation on metabolism and SOD activity of *Macrobrachium nipponensis*. Journal of Hebei University (Natural Science Edition), 27:537-540.

Zhang, W.H., X.Q. Xu, and C.Q. Qiu, 2001. Advance in study on microcystins in aquatic environment. Research of Environmental Sciences, 14(2):57-61.

Zhang, Y.L., S.Y. Wang, G.M. Liu, Y. Chen, and X.H. Zou, 2005. Variation of phenoloxidase activity affected by hemocyanin in shrimp *Penaeus vannamei*. Journal of Fishery Sciences of China, 12:402-406.

Zhao, F., P. Zhuang, and L. Zhang, 2006. The influence of salinity acclimation on activity of Na^+/K^+-ATPase in branchial epithelium, concentration of ions and osmolarity in serum of *Acipenser schrenckii* . Journal of Fisheries of China, 30:444-449.

Zimba, P., L. Khoo, and P.S. Gaunt, 2001. Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. Journal of Fish Diseases, 24:41-47.

Zimba, P.V., A. Camus, and E.H. Allen, 2006. Co-occurrence of white shrimp, *Litopenaeus vannamei*, mortalities and microcystin toxin in a southeastern USA shrimp facility. Aquaculture, 261:1048-1055.

Zurawell, R.W., H. Chen, and J.M. Burke, 2004. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. Journal of Toxicology and Environmental Health, Part B, 8(1):1-37.

Identifying Best Practices to Improve the Giant River Prawn Industry in Thailand

Production System Design and Best Management Alternatives/Activity/09BMA06UM

Yuan Derun
Network of Aquaculture Centres in Asia-Pacific
Bangkok, Thailand

James S. Diana and Vicki Schwantes
University of Michigan
Ann Arbor, Michigan, USA

Liu Liping
Shanghai Ocean University
Shanghai, China

ABSTRACT

In 2005, the University of Michigan project conducted surveys of prawn farming in Thailand, with intent to understand the environmental impact. The end results showed that prawn farming was lucrative for farmers, but there were major concerns about eutrophication of water sources, overuse of feed, and other environmental impacts symptomatic of over-intensity of prawn production. As a follow-up to that evaluation, this August, we brought together a group of managers and farmers and planned to review the status of prawn farming and educate them on how to minimize the environmental impacts from farming practices. As the workshop progressed, we were surprised to find that prawn farming had changed dramatically over the past six years. The typical prawn farmer had significantly reduced stocking density, tended to use no exchange water systems for production, and reduced total yield while achieving a higher standard product from the grow-out systems. As a result, the concerns about eutrophication and overfeeding had largely disappeared over that six-year period.

To our surprise, prawn farmers in Thailand had willingly changed their practices to a very substantial degree. In 2005, 96% of all farmers practiced intensive monoculture. While we were unable to conduct a similar survey with statistical methodology in the 2011 workshop, reports at the workshop indicated that 80% of farmers today used polyculture instead. The common polyculture was with *Macrobrachium* (about 6 pieces per square meter) and white shrimp *(Litopenaeus vannamei)* (about 12 pieces per square meter) in fresh water. This is in comparison with monoculture of prawns, which was done at about 40 pieces per square meter; so effectively, the overall density had decreased by at least one-half. Similarly, in 2005, feeds were often handmade and were of low quality with many fine particles, while in polyculture, commercial feeds were used, which are better controlled for quality. Feeding rates are now evaluated using feeding trays. Water exchange in 2005 was about 60% per pond per week, while currently, water is exchanged at a much lower rate, and most of that water is retained. These changes have occurred in part because of the adoption of the GAP standards for shrimp and applying them to prawns, and in part because of the move by the Thai Department of Fisheries to help farmers become more environmentally aware, as well as more profitable. In the new aquaculture system, most farmers rely on freshwater culture of white shrimp at low density for their basic income, and then the culture of prawn at even lower densities of prawns for supplementing their income because of the high market value.

INTRODUCTION

Aquaculture has a major role of producing food for both export and local markets. The case of the giant river prawn *(Macrobrachium rosenbergii)* is interesting in the region of Southeast Asia, particularly Thailand. Prawns are very popular food items in this region and, as such, contribute largely to the local

economy. In 2008, the total production of prawns in Thailand was approximately 32,000 metric tons, worth about $131 million. The production of prawns has maintained itself at around 30,000 tons since 2003, while the value has increased dramatically – almost doubling during that same time period. Clearly, the quality of the crop and the value per pound has increased over that time period. Prawns grown in Thailand are entirely marketed locally, with no export reported. As such, they are not necessarily good candidates for certification or market forces driving more sustainable production for several reasons. First, Thais already understand and value aquaculture. Second, local consumption eliminates organizations, such as importers from Europe or America, having any say in the standards for growing prawn. And third, local consumers in Thailand are more concerned about price than environmental performance.

In 2005, Schwantes et al. (2009) conducted surveys of prawn farming in Thailand, with intent to understand the environmental impact. The end results showed that prawn farming was lucrative for farmers, but there were major concerns about eutrophication of water sources, overuse of feed, and other environmental impacts symptomatic of over-intensity of prawn production. As a follow-up to that evaluation, we conducted a workshop in August 2011 intending to convince prawn farmers to undertake more sustainable production practices. We brought together a group of managers and farmers, reviewed the status of prawn farming, and educated famers and outreach personnel on how to minimize the environmental impacts from farming practices.

RESULTS

The workshop was held from 8-10 August 2011 in Bangkok, Thailand. A list of participants is provided in Table 1. The meeting agenda is shown in Table 2.

As the workshop progressed, we were surprised to find that prawn farming had changed dramatically over the past six years. The typical prawn farmer had significantly reduced stocking density, tended to use no exchange water systems for production, and reduced total yield while achieving a higher standard product from the grow-out systems. As a result, the concerns about eutrophication and overfeeding had largely disappeared over that six-year period. Farmers began following the Thailand GAP (good aquaculture practices) standards for Thai shrimp. The shrimp GAP includes ten requirements that are either major (must be adhered to), minor (requires 70% compliance), or recommended (requires 60% compliance). These include major categories of: farm location, farm management, use of drugs, effluent and sediment management, energy use, farm sanitation, harvest and post-harvest handling, labor and welfare, social and environmental responsibilities, and record keeping. While the system is voluntary, GAP standards have been adopted by a large number of shrimp farmers in an effort to improve the sustainability of their operations. Prawn farmers have adopted the same practices in absence of specific prawn standards.

To our surprise, prawn farmers in Thailand had willingly changed their practices to a very substantial degree. In 2005, 96% of all farmers practiced intensive monoculture. While we were unable to conduct a similar survey with statistical methodology in the 2011 workshop, reports at the workshop indicated that 80% of farmers today used polyculture instead. The common polyculture was with *Macrobrachium* (about 6 pieces per square meter) and white shrimp (*Litopenaeus vannamei at* about 12 pieces per square meter) in fresh water. This is in comparison with monoculture of prawns, which was done at about 40 pieces per square meter in 2005; so effectively, the overall density had decreased by at least one-half. Similarly, in 2005, feeds were often handmade and were of low quality with many fine particles, while in polyculture, commercial feeds were used, which are better controlled for quality. Feeding rates are now evaluated using feeding trays, mainly focused on the shrimp crop. Water exchange in 2005 was about 60% per pond per week, while currently, water is exchanged at a much lower rate, and most of that water is retained. These changes have occurred in part because of the adoption of the GAP standards for shrimp and applying them to prawns, and in part because of the move by the Thai Department of Fisheries to help farmers become more environmentally aware, as well as more profitable.

In the new aquaculture system, most farmers rely on freshwater culture of white shrimp at low density for their basic income, and then the culture of prawn at even lower densities for supplementing their income because of the high market value. As a result, their overall production has stabilized over the past six years, while the value of their crop has increased dramatically, and the environmental impact of their growing system has decreased dramatically. Assuming the reports presented at our workshop reflect what is actually happening throughout the industry, this is a win-win situation, with prawn culture remaining more profitable, while improving its environmental performance. Perhaps the most interesting portion of this impact was the rapidity of this change; in about six years, the industry has changed dramatically from one dominated by intensive monoculture, to one using more semi-intensive polyculture. Both systems are very profitable, but the current system appears to be more so because of the lower impact of diseases and the high value of large prawns, which grow more readily in the lower density culture systems.

DISCUSSION

The results of the workshop were surprising. Yuan Derun coordinated the workshop, inviting 14 farmers, as well as 17 researchers or government managers of aquaculture systems. Because the workshop was located in Bangkok, most farmers were from Suphanburi or Ratchaburi, two provinces near Bangkok. However, their views almost certainly reflect those of the industry, as those two provinces are the major producers of prawns in Thailand.

Overall, the workshop was a success in so many ways. From the AquaFish CRSP perspective, major changes in prawn culture have occurred in Thailand as a result of increasing scrutiny for environmental performance. The aquaculture programs continue to be profitable –even more profitable than before – but at the same time, much more environmentally sustainable. Intensive efforts by the Thai Department of Fisheries, various research groups including NACA (Network of Aquaculture Centers in Asia-Pacific) and the AquaFish CRSP, and the results of our previous study led this movement toward more sustainable production. More than anything, this change demonstrates the importance of sustainability to aquaculturists in Thailand and bodes well for the future of aquaculture in that region.

LITERATURE CITED

Schwantes, V.S., J.S. Diana, and Y. Yi, 2009. Social, economic, and production characteristics of giant river prawn *Macrobrachium rosenbergii* culture in Thailand. Aquaculture, 287:120-127.

Table 2. The agenda for the workshop on prawn aquaculture in Thailand.

Time	Activity	Lead
	August 8	
08:30 - 09:00	Registration/arrival of participants	
09:00 - 09:10	Welcome address	DoF DG; NACA DG
09:10 - 09:20	Remarks from the project PI	Prof. James S Diana
09:20 - 09:45	Presentation- Introduction to the workshop	Mr. Yuan Derun
09:45 - 10:00	V. Participants introduction	
10:00-10:30	GROUP PHOTO, Coffee/Tea Break	
	Plenary Session: Status review – Chair: Profs. Sena De Silva and C. V. Mohan	
10:30 – 11:30	Technology development in Improving Sustainability and Reducing Environmental Impacts of Aquaculture Systems	Prof. James S Diana
11:30 – 12:30	International principles for responsible aquaculture practice, BMPS and farmer clusters development	Prof. C. V. Mohan
13:30 – 14:30	Thai GAP and CoC	Dr. Putth Songsangjinda
14:30 - 15:30	Thailand Prawn production – 2005 survey report	Dr. Vicki S. Schwantes
	August 9 – Thematic Groups	
08:30 – 09:00	Thematic groups: Assignments of group members, leaders, rapporteurs and materials	Mr. Yuan Derun
09:00 – 12:30	Group work	Group leaders
13:30 – 15:00	Group presentations	Group leaders
	Working Group Synthesis	
15:30 – 17:00	Drafting the fact sheet on Better Management Practices for Prawn Hatchery and Grow-out Production	Chair: Prof. James Diana Synthesis working group
	August 10 - Plenary Session II: Better Management Practices for Prawn Hatchery and Grow-out Production Chair: Dr. Putth Songsangjinda	
09:00 – 10:00	Presentations on output of synthesis working group	Prof. James Diana Mr. Yuan Derun
10:30 – 11:30	• Suggestions for modification • Issues on implementation • Suggestions to follow up	The Chair
11:30 – 12:00	Thanks and Closing	NACA DG DoF reprehensive Prof. James Diana Other guests

Assessment of AquaFish CRSP Discoveries

Production System Design and Best Management Alternatives/Study/09BMA07OR

Steven Buccola and Lin Qin
Department of Agricultural and Resource Economics
Oregon State University
Corvallis, Oregon, USA

Rolf Fare
School of Public Policy
Oregon State University
Corvallis, Oregon, USA

ABSTRACT

Quantitative methods, combined with representative case studies, are used here to examine relationships between inputs and outputs in AquaFish investigations. The Data Envelopment (DEA) methods we employ to examine the 2007 – 2011 training investigations reveal wide differences in the efficiency with which training is produced. While some of that disparity likely can be explained by cross-investigation differences in such factors as travel distance and training intensity, some of it probably stem from unobserved variations in the manner in which US and host-country cost shares are allocated.

Regression instead of DEA methods are used to examine AquaFish's 2009 – 2011 research-oriented investigations. The Bayes value of sample information – namely the utility an investigation affords to a private or public decision-maker – is used in these regressions as the knowledge measure. The functional form we assume allows one to observe each research input's effect on the investigation's mean surprise and sampling accuracy as well total new knowledge. We find that investigation team size, team education, and distance to study site have moderate impacts on the mean surprise generated. Statistical accuracy is higher in experimental than in survey studies. Research outcome dimension and investigation topic area have weak implications for an investigation's mean surprise but strong implications for its accuracy. Returns to investigation scale are below unity, implying smaller-scale investigations tend to be more efficient than larger ones are. With some exceptions, these findings are broadly consistent with our assessment of the 2007 – 2009 research investigations.

INTRODUCTION

In research, training, and outreach programs like AquaFish CRSP, it often is useful to compare program outputs with inputs, providing an idea of how effective the program resources have been in producing new knowledge and pointing to better resource allocations. The present Investigation of the Research Discovery and Impact Assessment Project focuses on just such an assessment for the AquaFish CRSP. Its objective is to develop improved methods for assessing the discoveries arising from AquaFish CRSP experiments, studies, and activities, and to apply the methods to demonstrate how AquaFish has produced new knowledge and human capital. In particular, it examines the AquaFish discovery and training successes themselves as opposed to the wider impact those successes might have on the fish-farming industry and environment. To distinguish between these two purposes, we refer to the present Investigation as dealing with program *discovery*, and to Investigation #2 of this Project as dealing with program *impact assessment*.

AquaFish discoveries are approached here in two ways: (a) quantitative comparisons of investigation discoveries with the inputs used to achieve them, and (b) a series of seven brief case studies, one in each of the seven other AquaFish projects. The quantitative analyses reported here concentrate on only the AquaFish Phase II (2009 – 2011) investigations. Quantitative analysis of Phase I (2007 – 2009) investigations is provided in our accompanying Synthesis Project Final Report.

The purpose of the case studies is to provide insights into selected AquaFish investigations that may not be apparent from the AquaFish final reports or other publications. The cases were selected for their diversity and informativeness rather than "success." Although each story is unique, the case presentations follow, for the sake of comparability, an identical reporting format: a depiction of the problem tackled, the study's social and physical setting, the investigators' strategy in solving the problem, obstacles overcome or not overcome, and study results and likely impacts.

In contrast to the case studies, which seek to show the differences among AquaFish efforts, our quantitative analysis draws implications common to a group of investigations. The quantitative approach for examining AquaFish's research-type (experiment or study) investigations differs from the one we take for the training-type (activity) investigations. In both, we show how project inputs like total expenditure and science person-hours have led to project outputs. For the research investigations however, we construct a new Bayesian measure of knowledge output, while for the training investigations we take the standard approach of representing output as the number of training hours delivered. Analysis of research investigations employs parametric (statistical) estimates of a knowledge production function, while analysis of training investigations employs non-parametric (statistic-free) methods which identify the distance between each investigation and technically efficient performance.

In the following, we first take up the quantitative analysis of AquaFish's research-type investigations. We then turn to quantitative analysis of the training-type investigations, and finally to the case studies.

Analysis of Research-Type Investigations

INTRODUCTION

The wide variety of AquaFish research investigations creates a serious challenge to their quantitative assessment because it complicates the identification of valid correlations between knowledge-gained and resource-expended. Variety is found among research investigations' methods (experiment vs survey), types of findings (profitability, human health, ecosystem quality), and technological and cultural settings. That heterogeneity exacerbates the difficulties already present in AquaFish program assessment, in particular the data and conceptual problems of distinguishing program influences from other factors affecting the fish-farm or training setting. Much of our effort therefore was devoted to dealing with, and taking advantage of, cross-study heterogeneity.

In particular, investigations differ widely in their targeted outputs. Some concerned with yield-improving management practices, for example, focus on weight-gain enhancement while others focus on survival rate or fertility enhancement. The latter in turn are incommensurable with the water-quality or market improvements in other investigations. Furthermore, aquacultural research typically involves not only a variety of research treatments but a variety of outcomes per treatment. An examination of alternative fish feed rations, for instance, may be concerned with the survival rate, feed conversion, final body weight, and flesh quality associated with each ration. And a statistical survey of fish exporters may inquire about both desirable fish length and harvest date. This is because each such outcome affects profitability in a separate way. For all these reasons, cross-study assessments like the present one require not only a single but a flexible output metric.

Essential for this purpose is that the metric be expressed in terms of percentage changes, relative either to the output the researcher had anticipated or to that prevailing in the local area before the investigation began. Because percentage changes are always comparable with one another, they transcend differences among study outcomes. However, the metric must satisfy an additional requirement. Some investigations fail in the sense that the hypothesized improvement – a better water purification scheme, say – does not materialize. The failure does not, however, imply that expenditures have been wasted: the disappointment was valuable in pointing to more fruitful research directions (CGIAR Science Council 2009).

In order to capture the entire scope of knowledge a research study produces, we must cast it in terms of the information the study is *expected* to generate. For example, suppose researchers anticipate that a new Tilapia seed-production method will boost pond yields, but find in the course of their research that it instead reduces yields. The study nevertheless was a success insofar as it updated our knowledge of pond yield probabilities, expressed as a shifting or narrowing of the probability distribution of yield outcomes in the presence of alternative treatments. Our research discovery assessment consists in comparing these outcome distribution changes with the investigation inputs – such as expenditures, human capital, and effort – that have made the changes possible.

To compare outputs with inputs in this way, we regard each investigation as a production unit that uses inputs like money and personnel to produce discoveries (Buccola, Ervin, and Yang 2009; Xia and Buccola 2005). Every experimental investigation examines several alternative treatments, and every survey study examines several respondent sub-groups. Furthermore, a given experimental treatment gives rise to a multitude of findings or outcomes such as weight gain and feed/gain ratio, and a given survey involves a variety of questions. For each of these two reasons, we are able to examine a number of input-output combinations in each investigation and AquaFish research cycle. Pooling such combinations across all investigations in a given cycle (2007 - 2009 or 2009 - 2011) or Topic-Area category allows a comparison of research discoveries with research inputs in that investigation stratum.

A Bayesian Measure of Scientific Knowledge

A production function approach to research discovery assessment may be represented as

$$K = f(\mathbf{X}) = f(X_1, X_2, \ldots, X_I) \tag{1}$$

where K is a measure of the knowledge discovered in the investigation and $\mathbf{X} = (X_1, X_2, \ldots, X_I)$ is the vector of the study's I inputs. Estimates of equation (1) can be used to compute derivatives $\partial K / \partial X_i$, showing the effect on knowledge output K of changes in the study's i^{th} input. Because many study inputs can simultaneously be controlled in this fashion, equation (1) therefore is a foundation for assessing and improving research management and design.

Knowledge Outputs

An important aspect of our modeling is the manner in which the before-study ("prior") and after-study ("posterior") probabilities of outcomes K – like mortality rate – are obtained. Well-developed methods are available for eliciting investigators' prior probabilities of their discoveries (Stael von Holstein 1970). The methods involve casting the probability questions in the context of the study's institutional and technical environment. The corresponding posterior probabilities are obtained from the investigation's statistical results, in an experimental study embedded in the ANOVA analysis of the experimental outcomes and in a statistical survey in the means and variances of the survey responses.

Bayesian methods are a rigorous and self-consistent way of updating such probabilities as the investigation proceeds (Schimmelpfennig and Norton 2003, Carlin and Louis 1996, Press 1989). Unlike classical statistics, which allow inferences about a population, Bayesian statistics allow inferences about how much *new* knowledge has been gleaned from a research effort. In the present Investigation, we develop a new Bayesian approach for constructing the measure of knowledge-gained, and use it to assess output-input relationships in AquaFish research (experiment or study) investigations.

To summarize the Bayesian approach to measuring knowledge, let *Y* be the percentage improvement in a particular study outcome such as pond quality, and *Z* the experimental performance (sample information) of the pond-cleaning technology the researchers are studying. The likelihood that experimental outcome *Z* will occur depends on study inputs **X** and on random events ε; that is, $Z = Z(\mathbf{X}, \varepsilon)$. Bayes Theorem says the probability the investigator assigns to a particular pond-quality improvement is, once the experiments have been completed,

$$p[Y \mid Z(\mathbf{X},\varepsilon)] \quad \propto \quad p(Y) \cdot p[Z(\mathbf{X},\varepsilon) \mid Y] \tag{2}$$

where $p(\cdot)$ is probability, $\mid$ indicates the term following is held constant, and $\propto$ means "is proportionate to." Expression $p(Y)$ is the investigator's "prior" (pre-study) estimate of the chances that pond-quality *Y* will occur. Equation (2) thus says that, after the experiments have been conducted, the probability of pond-quality *Y* is proportionate to the probability of pond-quality *Y* originally anticipated times the probability (often called the "likelihood") that experimental outcome Z would be obtained in the presence of pond- quality *Y*. The left-hand side of equation (2) is called the "posterior" (post-study) probability of pond improvement. We will not be directly concerned in this report with the likelihoods of experimental events, but only with the prior and posterior probabilities of outcomes like pond quality.

The usefulness to us of Bayes' Theorem (2) is that it generates the very research knowledge measure *K* we discussed earlier. The underlying principle is that a research study's output can be expressed in terms of the useful information the study is expected to generate. To see this, suppose an oyster producer is faced – in the presence of her present oyster management practices **X** – with a decision *d* about how many oysters to promise to deliver next month at quality grade A. If she later delivers them at lower than the promised grade, her quality reputation will suffer; if at higher than the promised grade, she will not be prepared to market them at the proportionately higher price. Her profit or utility, that is, rises with her accuracy in predicting quality grade. Oyster management research improves such prediction accuracy – in proportion, in fact, to the difference which the research generates between the prior and posterior probability of oyster quality A.

In particular, the gain the oyster producer enjoys from basing her marketing decisions on posterior probability $p(Y \mid Z)$ rather than prior probability $p(Y)$ is reflected in the *value of sample (research-produced) information* **Z** (Winkler 1972):

$$VSI(d, \mathbf{Z}) \quad = \quad E''[U(d'' \mid \mathbf{Z})] \quad - \quad E''[U(d' \mid \mathbf{Z})] \tag{3}$$

where d' is the optimal decision in the presence of prior information only, d'' is the optimal decision in the presence of both the sample **Z** and prior information, and double-primes on expectations indicate they are taken with respect to the posterior (**Z**-present) information. Equation (3) is the difference between: (i) the utility the farmer expects to enjoy when promising the quality grade (d'') that is optimal in the presence of the sample information, and (ii) the utility she expects to receive when promising quality grade d' that had been optimal before sample **Z** was drawn, *both* expectations being computed in the

presence of the sample information. Thus also, the value of sample information is the disutility – computed from the *post-research vantage point* – the farmer suffers if deprived of the research study.

Research samples **Z** come at a cost, namely the expense of the study's inputs **X**. That is, we can generalize equation (3) by writing $\mathbf{Z}(\mathbf{X})$ in place of **Z**. And because the research knowledge *K* discussed above is precisely the value of the sample information which the research produces, we can write

$$(4) \qquad K = VSI(d, \mathbf{Z}) = E''\{U[d'' \mid \mathbf{Z}(\mathbf{X}, \varepsilon)]\} - E''\{U[d' \mid \mathbf{Z}(\mathbf{X}, \varepsilon)]\} = f(\mathbf{X}, \varepsilon)$$

where ε is random, unobservable study input and *f* is a production function. Expression (4) shows how study inputs are used to generate the sample information which improves farmers' prediction accuracy and hence management decisions, and on the basis of which his expected utility rises above what he faced with his inferior, pre-research information.

Importantly, knowledge measure (4) is a function only of the difference between $p[Y \mid Z(\mathbf{X}, \varepsilon)]$ and $p(Y)$, the after- and before-study predictions of a given fish-quality outcome. Because, thanks to the researcher's new sample information, the probability distribution of the former will never be less accurate than the distribution of the latter, knowledge-gained measure *K* will *never be negative.* Thus, although some research projects fail in the sense that a hoped-for technical improvement – enhanced pond yield, say – does not materialize, they can never fail to provide useful information. Even the disappointment was valuable in pointing to fruitful research directions (CGIAR Science Council 2009).

Functional Form

A number of functional forms are available for specifying fish-farmer utility *U* in (3) and (4) . We adopt here the mean absolute deviation (MAD) form $U(d, Y) = |Y - d|$ in which utility is proportionate to the absolute difference between a random outcome and its prediction (Robert 2001). In the present context it carries two assumptions: (a) the farmer loses as much utility when fish quality turns out to be a given amount *below* his quality prediction as it does when it turns out to be a given amount *above*; and (b) that loss is proportionate to the difference between the predicted and actual quality. The realism of these assumptions might be greater for some outcomes, like fish mortality or pond dissolved-oxygen content, than it is for others.

With the MAD functional form, knowledge equation (4) becomes

$$(5) \qquad K = \left(\sum_i |Y_i - M_{PR}|\right)_{PO} - \left(\sum_i |Y_i - M_{PO}|\right)_{PO} = f(\mathbf{X}, \varepsilon)$$

where M_{PR} is the mean of the prior probability distribution $p(Y)$ of *Y*, and M_{PO} is the mean of the posterior distribution $p[Z(\mathbf{X}, \varepsilon) \mid Y]$. The first middle term in equation (5) is the mean absolute deviation of a sample fish-quality observation from the pre-research quality prediction, namely $d' = M_{PR}$. The second middle term is the mean absolute deviation of an observation from the post-research prediction $d'' = M_{PO}$ (*Y*'s sample mean). In the present analysis, an AquaFish investigation's knowledge output is modeled as the difference between these two farmer risks, each computed from the post-research probability distribution of quality outcomes.

Numerical decomposition of (5) shows it can, with high ($R^2 \approx 0.97$) accuracy, be expressed as

$$\ln K = A + a_1 \ln\left(|M_{PR} - M_{PO}|\right) + a_2 \ln\left(STD_{PO}\right) \quad (6)$$

where A, a_1, and a_2 are estimated constants and STD_{PO} is the standard deviation of the post-research fish quality distribution. That is, a research study's information contribution depends separately on: (i) the absolute difference between the pre- and post-research estimate of expected fish quality (the study-produced absolute shift in the fish quality expectation and hence the study's *mean surprise*), and (ii) the post-research sample estimate of the standard deviation of quality outcomes (the study's predictive success and hence *accuracy*). This decomposition allows us much flexibility in examining the impacts of study inputs **X** on knowledge output *K*, for it permits us to distinguish between how those inputs affect the shift in a fish-quality forecast as well as the accuracy of that forecast. Mean surprise and accuracy are, that is, separate aspects of a study's forecast effectiveness. Mean surprise tells us how close the average dart is to the bulls eye; accuracy tells us how close the darts are to one another.

Expressions $\ln\left(|M_{PR} - M_{PO}|\right)$ and $\ln\left(STD_{PO}\right)$ on the right-hand-side of (6) were estimated – for each AquaFish research treatment, outcome, and survey question – by taking 50 random draws of each the two parenthesized expressions in the middle of (5) and using them to compute M_{PR}, M_{PO}, and STD_{PO}. Because outcomes Y_i in AquaFish studies are expressed in a variety of units (kilograms of fish per hectare, micrograms of oxygen per liter of water, and so forth), we render them comparable to one another by dividing each by the mean of the 50 outcomes drawn. Each outcome or finding level such as mortality thus is equivalently expressed as its percent deviation from its own posterior mean. Thus also, this normalization reduces posterior mean M_{PO} to 1.0 in each experimental treatment, outcome, and survey question, so that M_{PR} is expressed as a proportion of M_{PO}.

METHODS

Knowledge Outputs: Elicitation and Construction

Data required for constructing knowledge output measures *K* and input measures **X** were obtained from the key host-country individual responsible for carrying out the investigation. Because there were 24 key investigators, communication with them was conducted largely through a single coordinator in each of the seven AquaFish projects. Coordinators were (by U.S. university of the respective project): Steven Amisah (Purdue University), Gertrude Atukunda (Auburn University), Remedios Bolivar (North Carolina State University), Wilfrido Contreras (University of Arizona), Eladio Gaxiola (University of Hawaii), So Nam (University of Connecticut), and Gao Zexia (University of Michigan). Fifty-five research-type investigations were conducted under AquaFish's 2007 – 2009 and 2009 – 2011 phases. Twenty-seven of these, examined in the present Report, were conducted during the 2009 – 2011 phase. Twenty-eight, examined in the authors' Synthesis Report, were conducted during the 2007 – 2009 phase.

Outputs *K* were elicited by way of two Output questionnaire forms, one for an experiment-type and another for a survey-type study. These forms are shown respectively in Appendixes A and B. As Appendix A indicates, the scientist in an experiment-type investigation is asked – for each treatment (e.g. feed ration) and each outcome of that treatment (e.g. feed/gain ratio or mortality) – to state: (i) her prior or pre-study probability p_L, p_M, p_H that each of three selected (*L*, *M*, *H*) *experimental outcome* levels would be attained; and (ii) the corresponding posterior probabilities as represented by the outcome's mean and standard deviation in the ANOVA results. The scientist in a survey-type investigation is asked – for each major survey question (e.g., export-optimal fish species or length) – to state: (i) his prior probability p_L, p_M, p_H that he would obtain each of three selected (*L*, *M*, *H*) *survey answer* levels; and (ii) the

corresponding posterior probabilities as represented by the mean and standard deviation of the respondent's answers to that question. The investigator often had to use judgment in identifying which experimental outcomes or survey questions to report. In some studies, for example, respondents are asked 30 to 40 survey questions, or numerous water chemistry factors collected. To avoid highly uneven sample sizes across investigations, scientists were asked to choose no more than five or six of the most insightful or strategic questions they asked.

The rationale for the correspondences established between these two questionnaires is clear. Each prospective outcome of each experimental treatment in an experiment-type investigation constitutes an answer to a question, and hence can be compared with the prospective answer to each major question in a survey-type investigation. An example of responses to a survey-type output questionnaire is provided in Table 1 for Investigation 07MNE04UM, which examined relationships between pond nutrient content, effluent quality, and harvest yields.

Eliciting prior probabilities requires motivating the researcher to think deeply about how he expects the study will later turn out, based partly on the literature he has read and his conversations with others, and partly on his experience. These judgments are best elicited by casting the probability questions in the context of the study's institutional and technical environment. They also require practice. Opportunity for that practice was provided to most of the AquaFish investigators at our project workshops and meeting in San Diego, Seattle, and Shanghai, where Bayesian statistical theory was explained and participants worked through examples of the two types of output questionnaire. Forty-four investigators and staff attended the day-long 2009 San Diego workshop, 17 attended the two-and-a-half-day 2010 Seattle project meeting, and seven attended the six-hour 2011 Shanghai workshop.

Several difficulties were encountered in prior probability elicitation. The first is that, because the present project was not built into AquaFish's original design, we were – with every 2007 – 2009 investigation and most of the 2009 – 2011 ones – unable to elicit prior probabilities before the study actually began. Under these circumstances, investigators could be asked only to remember, or equivalently imagine, what their priors were at the start of their work. This may have introduced some bias in their responses because preliminary study results can color the scientist's prospects for the final results. However, investigators were frequently made aware of this problem during the training sessions and told us they were able to guard against it. Presence of bias is tested statistically below.

Respondents were asked (Appendixes A and B) to state their prior probability that each of three outcome levels, rather than intervals of levels, would be observed in the statistical phase of their research. Such level-type question simplifies the respondent's task, as it is relatively easy to identify a low, medium, and high outcome level. However, because in continuous-distribution situations a non-zero probability cannot be assigned to an outcome level, the respondent in these situations would theoretically have had to propose three alternative outcome intervals rather than points. In many cases, and at our suggestion, she did assign her prior probabilities to outcome intervals. For purposes of forming the associated cumulative densities, we used the midpoints of these outcome intervals.

Knowledge Inputs

Table 2 shows the kinds of inputs **X** used in an experimental or survey investigation. Money expenditure and human capital may be regarded as the positive factors brought to bear on a study question. Research problem type and public infrastructure may in contrast be considered as obstacles which those resources must overcome in achieving research success. The latter essentially are "negative resources" and thus must be specified along with the positive ones.

AquaFish-financed expenditure in the first category include salaries and wages, travel, research materials like feeds and medicines, training materials, student-workers' tuitions, and publication expenses. Cost

share, observable only at the project level, includes materials and equipment, administrative overhead, and portions of investigator salaries. Human capital – the knowledge and skills embedded in the investigators themselves – is to some degree reflected in their salaries. But those reflections are imperfect and usefully supplemented by, for example, information about the research team's academic rank and years of experience. More expenditure or human capital would provide greater scope for solving the research problem and hence presumably boost knowledge production *K*. The kinds of inputs included in cost share unfortunately vary across projects, so interpretations of cost share's knowledge impacts are inherently ambiguous.

Experimental approaches may be more or less difficult to plan and manage than survey studies are, so our expectations of experimental controls' impacts on knowledge production are somewhat ambiguous also. One might, however, at least expect controlled experiments to bring lower sample variances than survey studies do, since experimental controls are designed for very purpose of reducing random noise. An investigation's outcome or findings dimensions likely differ among one another in understandability or accessibility – fish weight gain perhaps being more difficult to measure than water oxygen level. Similarly, an investigation's Topic Area category may influence research difficulty insofar as some topics may be less understood and more expensive to address than are others. But we do not have strong prior expectations for such outcome-wise or Topic-Area-wise effects. A list of AquaFish Topic Areas, organized for purposes of the present analysis into five broad categories, is shown in Table 3.

Finally, public infrastructure can influence knowledge output in many ways. Distance and the difficulty of travel to study site consume resources that otherwise could be expended on knowledge-generation at the site. Climate, culture, or other geographic factors can have their own impacts on research success, for example when traditions among a surveyed group influence the types of information they are willing to reveal. National-level factor prices like wage rates affect the purchasing power of both cash and in-kind expenditures (Heston, Summers, and Aten 2011).

Data Elicitation

For an experiment-type investigation, an observation in the present study consisted of a pair of prior and posterior probability distributions for each treatment and for each outcome of that treatment. In survey-type investigations, an observation consisted of such a probability distribution pair for each survey question reported. Data on research inputs (i), (iii, parts a & d), and (iv, part d) were obtained from AquaFish project proposals and quarterly, annual, and final reports. Information about (iii, parts b and c) was obtained from the key investigator by way of the Output Questionnaires (e.g., Outcome Target, col. 4; Prior Probability Distribution, cols. 5 – 6; and Notes, col. 9, in Appendix A). Data on inputs (ii) and (iv, parts a, b, and c) were obtained from the key investigator via an Input Questionnaire, Appendix C. Procedures for administering the Input Questionnaire were the same as those for the Output Questionnaire, discussed above.

Data collection ran from October 2010 to December 2011. Part of the collection took place during the 2010 Seattle project meeting and 2011 Shanghai workshop, allowing us to address technical or conceptual problems as they arose. At one or other of the San Diego, Seattle, or Shanghai meetings, we were able to work directly with nearly all 24 of the investigators who had a key role in an investigation we examined. This early trouble-shooting helped avoid potential misunderstandings and enabled mostly smooth data elicitation afterward. Subsequent data submissions indicating any misunderstanding, or flaws in our questionnaire design, were returned for re-working.

Model Specifications

Indexing knowledge-production model (1) in terms of the types of research inputs discussed above gives

(7) $$K_{hijk} = f(\mathbf{X}) = f(\mathbf{E}_{ih}, \mathbf{H}_i, \mathbf{T}_{ijk}, \mathbf{I}_i)$$

in which

$\mathbf{E}_{hi}$ is the vector of expenditures on the i^{th} investigation of the h^{th} project, in dollars per biennium;

$\mathbf{H}_i$ is the vector of human capital variables in the i^{th} investigation;

$\mathbf{T}_{ijk}$ is the vector of research problem types in the j^{th} treatment and k^{th} outcome of the i^{th} investigation;

$\mathbf{I}_i$ is the vector of public infrastructure variables in the i^{th} investigation.

Details of these specifications are provided in Tables 2 and 4. The correspondingly indexed form of equation (6) is

(8) $$\ln K_{hijk} = A + a_1 \ln\left(|M_{PR} - M_{PO}|\right)_{hijk} + a_2 \ln\left(STD_{PO}\right)_{hijk}$$

in which

(9) $$\ln\left(|M_{PR} - M_{PO}|\right)_{hijk} = f_1(\mathbf{X}_{hijk}, e_{1,hijk})$$

(10) $$\ln\left(STD\right)_{hijk} = f_2(\mathbf{X}_{hijk}, e_{2,hijk})$$

We therefore are able to estimate the latter two equations separately, and so can observe how research inputs affect research distribution shift or *mean surprise* (9) separately from how they affect research *accuracy* (10). We then combine them, *via* equation (8) and its α_1 and α_2 parameter estimates, to conclude how research inputs affect total knowledge output – the expected value of the sample information provided by AquaFish research.

RESULTS

Summary Statistics

The sample data are summarized in Table 5. Sample size in the 2009 – 2011 phase was 284. In the typical investigation, treatment, and outcome (experimental finding or survey answer), the researcher's prior outcome expectation was 0.171 (17.1%) higher or lower than the mean outcome or answer in the subsequent experiment or survey. That is, the researcher's expectations tended to be 17% "off" of what eventually happened, creating an average 17% mean surprise. The associated standard deviation (0.195) is slightly higher than the mean, implying a great deal of variation in how far a researcher's prior expectations missed the eventual mark. The distribution of these absolute surprises was skewed strongly to the right: mean surprises tended to cluster just above zero, the successively larger ones less frequent, and in only a few investigations very high.

The shape of the sample distribution of posterior standard deviations – measuring the spread or inaccuracy of research outcomes around their experimental or survey means – is similar to the distribution of mean shifts or surprises. Average experimental or survey outcome was 0.335 (33.5 percent) above or below its own mean. The 0.370 standard deviation of this accuracy across investigations, treatments, and outcome types reveals rather strong sample variation among the investigations' sample variances. And the distribution of the accuracies across investigations and outcomes was positively skewed. They bunched just to the right of zero, where standard deviations are low, the successively larger ones (with

higher standard deviations and hence less accurate) being continuously less frequent. Because our knowledge measure is a weighted sum of mean absolute surprise and variance, their sample coefficients of variation (standard deviation divided by mean) of 1.14 and 1.10 are adequate for examining the factors affecting AquaFish knowledge generation.

Average AquaFish-financed expenditure on an investigation was $90,645, and host-country and US cost shares respectively $82,438 and $175,353. The average research team consisted of about 10 individuals, the average investigator 32 years old with 17 years of education. Sixty-three percent of the research outcomes pertained to fish production (growth, feeding, mortality) 12% to water quality, 13% to marketing potential, and 11% to species development. Fifty-eight percent of investigations fell into a fish production topic area, 16% into marketing, 4% into technology adoption, and 22% into environmental problems. Seventy percent involved controlled experiments and 30% surveys. Average distance to experiment or survey site was 1134 kilometers; 62% percent of the roads to them were paved, 14% gravel, 24% dirt. Ninety-three percent of transportation to research site primarily involved auto or bus, 7% walking.

Sample variation of most of these research inputs is adequate for regression inference. For example, coefficient of variation of AquaFish cash expenditure is 0.39 and of team *FTE* 0.35. Lowest coefficients of variation in Table 6 are in the research team's average age (0.13) and education (0.06) and in host-country cost share (0.19). Coefficients of variation of the research outcome dimensions and topic-area categories are mostly above one. Relative variation in the proportion of investigations in which walking was the main mode of access to research site was particularly high (cv = 3.71).

Few of these variables are pairwise-correlated to the degree creating inference problems. Possible exceptions are that African projects came with especially high host-country cost shares (correlation 0.63 between HC cost share and African status). Scientist age and education are correlated 0.71. The number of full-time-equivalent (*FTE*) scientists in an investigation is significantly negatively (-0.52) correlated with average scientist age, suggesting investigations can somewhat be divided into those with many (and young and thus cheap) workers and those with few (and old and thus expensive) ones. Several of the transportation-mode and road-condition variables are correlated strongly.

The concern that several of the research outcome dimensions employed here might be correlated highly with some topic-area categories – because, for example, production and marketing appear in both lists – does not for the most part materialize. The strongest pairwise relationships are that marketing outcomes are correlated 0.59 with the marketing topic area, and species-development outcomes 0.67 with the environmental topic area. These generally low correlations are achievable because an investigation can fall into only one topic area but can examine a variety of outcome dimensions, such as both fish growth and water quality. Nevertheless, despite the typically moderate pairwise correlations in our sample, multicollinearity possibilities remain whenever a large number of explanatory variables are specified in a regression equation.

Because knowledge *K* depends partly on the scientist's subjectively determined prior expectation M_{PR} of the study outcome, one ought first check whether the reported expectations contain investigator bias. In particular, investigators may have tried to record a large mean surprise by reporting a prior mean they knew to be far from the eventual experimental or survey outcome. Examination of sample *algebraic* differences M_{PR} - M_{PO} in our data, however, suggest otherwise. Except for a small right tail, the distribution of these differences is approximately symmetrically distributed around zero. As a long-run average, that is, investigator research predictions tended to be accurate, an accuracy that strategic

expectations reports would have frustrated. The rather high mean *absolute* differences $|M_{pr} - M_{po}|$ in Table 5 show of course that investigators differed widely in their ability to predict eventual outcomes.

Factors Affecting Output: Mean Surprise

We address first the factors affecting mean research surprise, then those affecting research accuracy, then those affecting their weighted average constituting scientific knowledge. Estimates of research surprise equation (9), with the 2009 – 201 sample of 284 observations, are shown in Table 6. Sixteen outlier observations were dropped from the original 300 observations on account of their excessive influence on regression estimates. Column (2) gives the coefficient estimates and column (4) the *t*-statistics. Because both research surprise and the continuous research inputs are expressed in logs, coefficients of these Table 6 inputs are "output elasticities," the percent increase in surprise generated by a one-percent rise in the input. The coefficient of a categorical (zero-one) variable in Table 6 is instead the percent change in mean surprise when switching to the indicated condition from the condition's base group.

Statistical significances of the Table 6 effects are rather low, particularly compared with the corresponding effects in our analysis of the 2007 – 2009 AquaFish phase (see Synthesis Project Final Report). The reason likely is due to the difference in the sample sizes available for these two studies. We are able to explain about only one-quarter of the mean-surprise variation in either AquaFish phase (R^2 = 0.23 in the 2007 – 2009 phase, 0.25 here in the 2009 – 2011 phase). But the sample size available in the 2009 – 2011 phase is only 284, namely 33% less than the 424 in the 2007 – 2009 phase. Sample size substantially affects regression estimates' standard errors and hence the *t*-values indicating statistical significance. In situations like the present, that is, where quantitative explanation of a dependent variable is difficult, a large sample size is important for securing satisfactory confidence intervals around the regression estimates. Given the comparatively small sample in the 2009 – 2011 analysis, we are forced to employ a wider confidence interval in it for assessing inputs' mean-surprise effects.

All three continuous inputs in Table 6 had the implications for mean research surprise we had expected. Expanding a study's size by increasing the number of its full-time-equivalent investigators modestly but at the 10% significance level shifts the location of the research outcome's probability distribution. In particular, a one-percent *FTE* boost lifts mean surprise by 0.12 percent. The bigger the team, that is, the greater the mean surprise expected. The percentage boost given to mean surprise by raising the team's aggregate education is even greater, although with lower statistical significance. Raising team education by one percent lifts mean surprise by 0.4%, with significance at the 15% level (t = 1.49). Finally, reducing travel distance by one-percent would bring only 0.01% greater mean surprise, all else constant. That effect, too, is significant at only the 15% level (t = -1.44). Although very modest, it is consistent with our expectation that travel time reduces the time and energy available for creativity and innovation.

While nonsignificant at usual confidence limits, experiment-type investigations bring, all else equal, an average 7.5% less surprise than do survey-type investigations (t = -1.11). This compares with the 13% lower mean surprise (t = -1.95) that they brought during the 2007 – 2009 phase. These results are understandable. The possibility of analyzing a problem with experimental controls normally is found where the scientist is relatively familiar with the problem's stochastic environment. That familiarity in turn implies one would not normally expect the research project to greatly change the investigator's *expectations* of a study outcome. Survey methods, in contrast, usually are more exploratory, to which investigators resort when the environment is comparatively unknown. Such expectations weakness is conducive to marked *changes* in the expectations as the study proceeds.

At normal confidence levels, type of research finding does not affect mean surprise once the remaining factors are accounted for. As in the 2007 – 2009 analysis for example, outcomes relating to fish production generated no greater surprise (t = - 0.84) than do those in the water-quality base group. Unlike

in 2007 – 2009, marketing findings' tendency to bring greater surprise (7% more, compared with 2007 – 2009's 28%) 2007 – 2009) has *t* statistic only slightly above unity. The latter weakness may be affected by the stronger correlation (0.60 vs 0.36) that marketing outcomes bear to the marketing topic area in 2009 – 2011 than they do in 2007 – 2009. In any event, it is important that we not confuse the marketing-outcome effect on knowledge production with the experiment-*vs*-survey effect. Survey methods were used extensively to answer water-quality and fish-population questions as well as marketing questions, and correlation between experiment-*vs*-survey) and the marketing outcome dimension in 2009 – 2011 investigations is only -0.34, well below the Belsley-Kuh-Welsh (1980) plus-or-minus-0.80 threshold for a problematic pairwise correlation between explanatory variables.

As in 2007 – 2009, an investigation's topic area itself also appears to have no significant effect on the mean surprise it generates. Table 6 shows the estimated mean-surprise differences between the production, human health, and environmental topic categories and the marketing base group, once the other factors are accounted for. All differences are statistically non-significant, both from the base group and from one another. Finally, investigations in Asia have, after controlling for the other factors in this analysis, brought 33% less mean surprise than did those in Africa. Although that result is statistically significant, its interpretation is ambiguous. Asian investigations may have involved better-understood problems than Africa ones did, bringing lower prospects for fundamental surprise.

Research mean-surprise model R-square is 0.25. We are able to explain about one-quarter of the variation across treatments, outcomes, and investigations in 2009 – 2011 research-induced mean probability distribution shift. The modesty of this proportion is to be expected. The investigator insights needed to fundamentally shift our understanding of a research problem are relatively ineffable and not easily explained with measurable inputs. That is not, as we shall see next, true for the research accuracy dimension.

Factors Affecting Output: Statistical Accuracy

Consider now (Table 7) the corresponding estimates of research accuracy equation (10), showing research inputs' effects on the standard deviations of the experimental or survey-question outcomes and thus on the precision with which these outcomes can be predicted or explained. For brevity, we often will refer to these standard deviations as variances. Because precision varies inversely with variance, factors with negative effects in Table 7 are those contributing positively to precision and thus to knowledge.

All but two of the factors in Table 7 is statistically significantly different from zero at least close to the 5% level. One with nonsignificant effect is team *FTE*. A one percent *FTE* rise brings only 0.06% higher predictive accuracy in the investigation's experiments and surveys, and the *t*-statistic is below unity. This contrasts with the highly significant 0.13% greater accuracy in the 2007 – 2009 phase. Unexpectedly, greater research-team education is associated with higher sampling variance: a one-percent rise in the team-members' average formal education lifts outcome standard deviation by 0.71%. We had expected contrarily, and indeed found in the 2007 – 2009 investigations, that team education would reduce variances because better-educated teams should be better able to maintain the experimental controls which restrict random variation. At the ground level, these controls usually are in the hands of the team's most-junior members, whose education levels have a strong role in our mean team-education variable.

In 2007 – 2009, we had found that greater travel distance to study site leads to statistically significantly – but barely perceptibly – greater sampling variance and thus to lower accuracy, the greater distance presumably inhibiting study monitoring. Rather consistent with that result, distance's effect in Table 7 is not significant at all. And like in 2007 – 2009, investigator choice between an experimental and survey research design has a much more profound research-accuracy implication than distance has. Experiment-type investigations provide, all else constant, 60% lower outcome variance and thus more accurate study results than do survey investigations. This effect is highly statistically significant and intuitive. As noted

above, experimental controls are used for the very purpose of reducing study outcome variances below what could be had with statistical controls.

Table 7 shows that investigation findings relating to fish production, marketing, and species development in either experiment and survey studies have lower sample variances than do water-quality findings (the base group). These outcome effects are statistically significant at least at the 10% level and rather high in magnitude. Fish-growth findings are 28% more accurate, marketing findings 13% more accurate, and species-development findings 48% more accurate than water-quality ones are. (Corresponding percentages in the 2007 – 2009 investigations were 30%, 34%, and 54%.) It follows in the 2009 – 2011 data that fish-production findings are substantially more accurate than marketing findings are. And water-quality findings suffer from substantially more unexplained variability than the others do.

Unlike its effects on mean research surprise, an investigation's topic area has strong implications for research accuracy. Controlling for the other factors, investigations in the fish production, human health, and environmental topic areas bring significantly more accurate conclusions – in the sense of tighter sample distributions – than do those in the marketing area. Production topics bring 14% more accuracy, human health topics 50% more, and environment topics 20% more, than marketing topics do. (Corresponding percentages in the 2007 – 2009 data, also statistically significant, were 48%, 22%, and 47%.) These impacts do not appear to be confounded with the outcome-dimension ones discussed in the previous paragraph, despite that the sample correlation between marketing outcome and marketing topic area is 0.60. Many marketing-topic-area investigations involve, for instance, fish production and environmental findings, and many marketing findings are found in environmental and food-safety topic areas.

Our research accuracy model's R^2 is 0.65. We are able to explain about two-thirds of the statistical-accuracy variation among AquaFish's 2009 – 201 investigations, treatments, and outcomes. In the Synthesis report, we note were able to explain one-half the statistical-accuracy variation in the 2007 – 2009 data.

Implications for Knowledge Production

In the above two sections, we have estimated how AquaFish research inputs affect the means and variances, equations (9) and (10), of the scientist's findings. Mean-shift and variance – mean surprise and accuracy – in turn constitute knowledge production. We now aggregate the Table 6 and 7 estimates, by way of equation (8), to see how research inputs influence knowledge production itself. First we regress equation (6) on the sample data, obtaining:

$$(11) \qquad \ln K = \underset{(-16.97)}{-0.112} + \underset{(62.28)}{0.873}\ln\left(|M_{PR} - M_{PO}|\right) - \underset{(-10.90)}{-0.101}\ln\left(STD_{PO}\right) \qquad R^2 = 0.93$$

in which the left-hand term is the log of knowledge output, the second right-hand term is the log of mean shift in AquaFish findings, and the third right-hand term is the log of the standard deviation of those findings. Numbers in parentheses are *t*-statistics. Mean surprise is a positive element, and standard deviation (inaccuracy) a negative element, of knowledge. Given MAD utility functional form (5), mean surprise is – at the margin – substantially more important to knowledge-creation than accuracy is. In particular, equation (11) shows that mean surprise's knowledge weight is 0.873 / 0.101 = 8.6 times greater than accuracy's knowledge weight. Using these relative weights and Tables 6 and 7, we compute in Table 8 each research input's net knowledge effects. For purposes of the Table 8 calculations, we have set to zero any input effect that was statistically nonsignificant at the 15% level in Tables 6 and 7.

Table 8 shows that, by way of mean surprise alone, the net knowledge impact of expanding research-team *FTE* is (0.873) (0.121) = 0.106. In other words, by virtue of its boost to mean surprise, expanding team hours by one percent enhances knowledge creation by 0.11%. And because the team-size effect on statistical accuracy is nonsignificant even at a generous confidence level, size's mean-surprise effect is its total effect. Thus, a one-percent team expansion boosts new knowledge by a net 0.106 – 0 = 0.11%. Science labor has a positive though moderate marginal impact on scientific knowledge.

On account of its (modestly) statistically significant boost to mean surprise, a one-percent improvement in the team's education lifts knowledge creation by (0.873) (0.397) = 0.346%. On account of its surprising exacerbation of sampling variance, education impairs knowledge creation by (- 0.101) (0.709) = - 0.072%. The net new-knowledge impact of boosting education by one percent is thus 0.27%. Despite, that is, its quixotic negative influence on research accuracy in the 2009 – 2011 data, education has a positive influence on scientific knowledge.

Finally, as we have seen in Tables 6 and 7, reducing distance to study site has little effect on either mean surprise or statistical accuracy. Its net-knowledge effect by way of mean surprise is only (0.873) (0.01) = 0.009, and by way of statistical accuracy is nonsignificant. Thus, as Table 8 shows, distance has only a 0.01 knowledge elasticity in the 2009 – 2011 investigations. Reducing distance one percent expands scientific knowledge by 0.01%. This is little different from distance's modest 0.03 elasticity in the 2007 – 2009 investigations.

Following the same procedures, Table 8 shows, holding the other factors constant, that fish-production, marketing, and species-development findings contain respectively 2.8%, 7.6%, and 4.9% more new knowledge than water-quality findings do. These positive margins are smaller than the respective 4.6%, 31.2%, and 82.9% margins encountered in th
e 2007 – 2009 investigations. In the present 2009 – 2011 data, they come nearly entirely from the findings' accuracy rather than mean-surprise aspect.

As in 2007 – 2011, topic-area category was shown in Table 6 to have had no statistically significant bearing on 2009 – 2011's mean surprises. Hence, topic area's influence on knowledge creation is again just its influence on accuracy weighted by accuracy's implication (- 0.101 in the 2009 – 2011 data) for net new knowledge. Production, health, and environmental topic areas thus bring in Table 8 an average 1.4%, 14.6%, and 2.0% more new knowledge than does the marketing topic area, all other factors – including the finding dimension – accounted for. The corresponding margins in 2007 – 2009 were 7.3%, 3.4%, and 7.2%.

Returns to Scale

The sum of an enterprise's continuous-input production elasticities constitutes the enterprise's returns to scale. Because we regard *FTE*, education, and travel-distance-reduction as inputs in a research enterprise, we therefore can compute returns to scale in the production of mean surprise (Table 6) as (acknowledging the effects are significant at only the 10% to 15% level) 0.121 + 0.397 + 0.010 = 0.528. Returns-to-scale below unity imply productive efficiency declines as enterprise size is expanded. Thus, scaling team labor time and education upward and travel distance downward would, while bringing more average surprise, do so with declining input efficiency. Undoubtedly, this reflects the poor replicability of the lead scientist's time and creative talent, which presumably are mostly responsible for the location shift of the outcome density function that provide mean surprise. However, 0.528 is substantially greater – implying a lower efficiency-loss rate – than the 0.093 returns-to-scale encountered in the 2007 – 2009 investigations.

Returns to scale in the production of research accuracy are negative in the 2009 – 2011 phase on account of education's unexplainably negative accuracy impact in Table 6. But even if education's 0.709 variance

elasticity is maintained, returns to scale in net knowledge creation remain positive in the 2009 – 2011 data. Weighting the statistically significant mean-surprise elasticities by their 0.873 knowledge weight and accuracy elasticities by their – 0.101 weight gives a net return-to-scale of 0.106 - 0.072 + 0.009 = 0.043. (In the 2007 – 2009 investigations, the net was 0.242.) In other words, returns to scale in 2009 – 2011 AquaFish knowledge creation were positive though strongly decreasing, implying steeply declining productive efficiency as investigation size grows. Adams and Griliches (1996) similarly find decreasing returns to scale in U.S. academic research. Decreasing returns signify a fixity in the research inputs excluded from the computation. Many such inputs clearly have not, from the evidence of our regressions' $R^2 s$, been captured in the present analysis at all. But others are present in the form of the research outcome dimensions and topic-area categories. Each confines what the investigator is examining and hence implies a constraint in its own right, limiting the possibilities for efficient scaling-up of research effort.

Thinking about the factors that were nonsignificant in a regression analysis can be as important as thinking about those significant, and several research inputs consistently had non-robust or nonsignificant effects in the present analysis. Because of their high correlation with other inputs, AquaFish dollar expenditures had unstable estimated impacts on knowledge output. U.S. and host-country cost share effects were unstable as well, partly because they are observed only at the project level and partly because the expenses they represent – salaries, materials, capital infrastructure – vary unevenly across project. Experiment and survey sample sizes, which vary by treatment, outcome dimension, and survey question as well as by investigation, ought by statistical theory to have a negative effect on posterior variances. In our own results they appeared to have no impact at all. Collaborator hours and education also were nonsignificant, and road- and transportation-type effects were non-robust. Stratification of the analysis by topic-area category required too many observations to be feasible.

CONCLUSIONS

We have statistically assessed the factors affecting the knowledge generated in AquaFish investigations, using the Bayesian value of sample information as the knowledge measure. A mean-absolute-deviation utility functional form allowed decomposing that knowledge into two independent effects: the difference (mean surprise) between the prior expectation of the research outcome and its posterior or statistical mean, and the outcome's statistical variance (accuracy). The former speaks to research's ability to shift the *location* of the probability distribution of possible study outcomes, and the latter to its ability to improve the *tightness* of that distribution. The decomposition permitted separate examination of research inputs' effects on these two distinct knowledge dimensions as well their effects on net or total knowledge.

We find, in 2009 – 2011 AquaFish research, strong evidence of decreasing returns to scale in the production of both research surprise and net knowledge creation. Likely, much of the surprise depends on the lead investigators' skills in question formulation and research strategy, which cannot easily be replicated with additional money inputs. Replicability and hence feasible investigation expansion is especially curtailed by the variety of topics and outcomes pursued in AquaFish research, which serve as a fixity which frustrates scale economy. It might be argued that topic variety is, in the present setting, socially more important than returns-to-scale are.

Analysis of Training-Type Investigations

Qian Wu, Oregon State University, assisted with this training-investigation analysis.

INTRODUCTION

Training investigations take approximately 35 percent of the AquaFish CRSP budget and may, like research investigations, be regarded as productive units (Charnes, Cooper, and Rhodes 1978). Measuring the efficiency of these investigations provides an important policy tool for USAID policy makers. The method of doing so in the present study gives information about: (i) each training investigation's relative technical efficiency, and (ii) how each investigation might allocate its inputs and outputs to attain full efficiency (Groot and Garcia-Valderrama 2006, Tone 2001).

METHODS

A sum-directional distance function is applied to our data to provide information about (i) and (ii). This distance function, introduced by Fare and Grosskopf (2010), allows investigations to reallocate their inputs and outputs to attain efficiency. Comparing efficient with observed allocations of inputs and outputs provides the decision maker information about policy actions that might improve total training budget use. The distance function also yields an efficiency indicator for each investigation, allowing it to be ranked with respect to performance.

We treat each AquaFish training investigation as a productive unity in the sense of Data Envelopment Analysis (Charnes, Cooper, and Rhodes 1978). Each unit, $k = 1, \ldots, K$, uses inputs $x^k = (x_{k1}, \ldots, x_{kn})$ to produce outputs $y^k = (y_{k1}, \ldots, y_{km})$. These observations form the reference technology toward which efficiency is calculated.

Fare and Grosskopf (2011), building on Tone (2001), formulated a sum-directional distance function as a measure of efficiency relative to the DEA reference technology. In particular, a training investigation's technical efficiency is specified as the following optimization problem:

$$
\begin{aligned}
\alpha_O = \max \quad & \beta_1 + \ldots + \beta_N + \gamma_1 + \ldots + \gamma_M \\
s.t. \quad & \sum_k^K z_k \, x_{kn} \le x_{on} - \beta_n, \qquad n = 1, \ldots N \\
& \sum_k^K z_k \, y_{km} \ge y_{om} + \gamma_m, \qquad m = 1, \ldots, M \\
& z_k \ge 0, \beta_n \ge 0, \gamma_m \ge 0, \\
& k = 1, \ldots K; \qquad n = 1, \ldots, N; \qquad m = 1, \ldots M
\end{aligned}
\tag{12}
$$

where z_k , $n = 1, \ldots, k$, are the intensity variables. The model reduces inputs by β_n ($n = 1, \ldots, N$) and increases output by γ_m ($m = 1, \ldots, M$). The optimal input to (12) yields α_0 for investigation "0" and shows by how many units inputs may be reduced or output raised. Because the intensity variables z_k , $k = 1, \ldots, K$, are restricted only to be nonnegative, the model exhibits constant returns to scale.

Figure 1 depicts this efficiency mechanism. Points A, B, and C are three observed investigations, all using inputs **x** to produce outputs **y** under constant returns to scale. Points A and B are on the best

practice frontier, meaning they cannot be improved upon. The optimal path to C's efficiency is to raise its outputs from Y_C to Y_D and decrease its inputs from X_C to X_D, arriving at D. MATLAB 7.0 is used to calculate the efficiency scores, and input and output changes achieving the best-practice frontier.

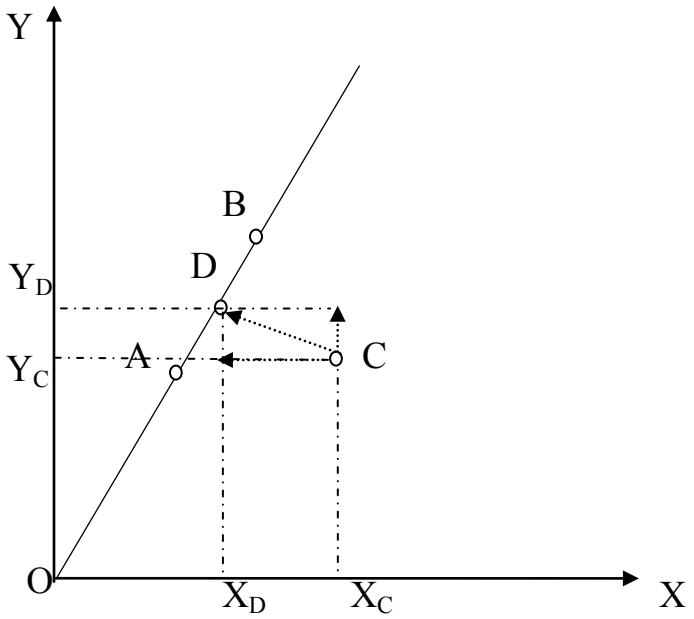


Figure 1. Technically Efficient and Inefficient Activities

AquaFish training investigations involved workshops, meetings, training sessions, and podcasts for a variety of participants, including farmers, technicians, researchers, and sometimes potential investors. They provided training in pond layout and design, stocking techniques, feeds and feeding, water management, processing and packing, and management. Conducting these programs required trainer time, training materials, transportation expenditures, and other inputs.

Investigation outputs are represented here by the number of person-training days provided. The modest number of training investigations prevents specifying a large number of alternative inputs. Four inputs are specified here:

(i) AquaFish expenditure, observable at the investigation level
(ii) US-institution cost share, observable at the project level
(iii) Host-country-institution cost share, observable at the project level
(iv) Number of workshops provided during the two-year project

The advantage of using expenditures and cost shares is that they represent most of the investigation inputs. The disadvantages of project-level cost shares is that they are not broken down by investigation; and the types of inputs to which they correspond differ across projects. The number of workshops can be considered an output measure; but it also is an input measure because it proxies for the set-up and set-

down costs of workshop organization. It is considered here to be an input, that is a drag on the number of training hours provided with given resources (although it also might be correlated with training quality).

Thirteen training-type investigations, collectively involving 26 workshops, were conducted during the 2007 – 2009 AquaFish phase. Nine training-type investigations, involving 28 workshops, were conducted during the 2009 – 2011 phase. Several investigations that comprised large conferences were considered non-comparable to the others and omitted.

RESULTS

Table 9 provides the results for the full 22-investigation data set. Quantities in the x_1, x_2, x_3, x_4 and y column are respectively the four observed inputs and the output. Corresponding columns with *d* subscripts show the technically efficient input levels, namely on the efficiency frontier as defined by the most-efficient investigations. The inefficiency (*DSDM*) measure in the right-hand column is the sum (divided by 10,000) of the investigation's observed input and output distances to the frontier. Hence, a given row in Table 9 indicates both the technical inefficiency and the changes that would correct it in the least-cost way.

An investigation provided, on average, 179 person-days of training to 105 trainees, that is 1.7 days of training per trainee. An average of 2.45 workshops were conducted, the majority short-term ones varying from one to three days. The mean number of trainees per workshop was about 43. AquaFish expenditures on these training investigations averaged $60,776. They differed dramatically from one investigation to the next. Mean host-country cost share was $95,716, and average US-institution cost share $149,562. Host-country cost shares varied substantially across investigations but US cost shares varied little.

We can see in the bottom rows of Table 9 that four investigations (two in the 2007 – 2009 AquaFish phase and two in the 2009 – 2011 phase) achieve, among all our observations, maximal relative efficiency in the sense of producing as many person-training days as possible with the resources at hand. Two of these most-efficient are in Latin America, one in Asia, and one in Africa. One is in technology adoption (TAP), one in production systems (BMA), and two in human health (HHI). In contrast, among the four most-inefficient investigations, two are in Latin America, one in Africa, and one in Asia; they cover a variety of topic areas. Considering the rest of the investigations also, there thus appears to be little association between apparent inefficiency and either location or topic.

But inefficiency ratios x_i^d / x_i in Table 9 are difficult to believe. They say, for example, that investigation 09BMA06UM could have produced the training hours it did with only 7% of the AquaFish expenditure it used. Some of the host-country cost share inefficiencies seem to be worse. Among the 18 inefficient investigations, average ratio of optimal to observed input is 26%. Evidently, the four most-efficient investigations at the bottom of the table are setting an impossible efficiency standard for the rest to follow. That is explainable either by supposing the top-four had enormous and nonreplicable cost advantages or that our cost data contain substantial inaccuracy. Because the AquaFish expenditures are indisputable, and the US cost shares do not differ much across projects, we think the reported host-country cost shares poorly reflect host-country contributions to the training projects, in turn skewing the inefficiency magnitudes.

As a robustness test, therefore, we eliminated Table 9's seven most-efficient investigations and re-ran the analysis without them. This allowed the most-efficient investigations to arise from among the remaining 15 in the sample. Results are shown in Table 10. Five of the investigations in Table 10 are on the efficiency frontier: one in Latin America, two in Asia, and two in Africa. Each focused on a different

topic area. The less efficient investigations likewise are in a variety of locations and topic areas. Again, therefore, no geographic area or training topic reveals any efficiency dominance. Indeed, the absence of any systematic effect of topic or regional location on efficiency appears to be an important conclusion from this work. However, optimal AquaFish expenditures and cost shares continue to be rather small percentages of the observed levels. Among inefficient investigations, ratios of optimal to observed inputs average 30%.

CONCLUSIONS

In this application of directional-distance modeling to AquaFish training activities, we find wide efficiency differences among investigations. The differences are so wide as to suggest that some expenditure data inaccurately represent training input levels. Our judgment is that the greatest source of inaccuracy is in host-country cost shares. Like US cost shares, they are reported only at the project level, so we do not know which portion actually were spent on the training activities. Nor do we know how they were distributed across the several countries in which a project was involved. Finally, there is some evidence of variation across projects in the *kinds* of inputs – salary, infrastructure, materials – the cost share was supposed to have defrayed. These variations become especially misleading in data envelopment methods like directional distance modeling because identities of efficient and inefficient investigations can be greatly affected by even a single large data inaccuracy.

Hence, we cannot place a great deal of weight on the Table 9 and 10 results. That conclusion suggests in its own right how valuable it would be for evaluation and programming purposes to have cost-share data that reliably reflects the types and magnitudes of program inputs. The results do, however, probably contain truth about the rank orderings (as opposed to the magnitudes) of the efficiencies in AquaFish training programs. We should keep in mind that at least some such inefficiencies are legitimate in the sense of reflecting road conditions, travel distances, hotel rates, and training program intensity. They do not necessarily reflect managerial error. As part of, but not reported in, our above quantitative study of AquaFish research investigations, we found that investigation expenditures are significantly explained by several infrastructural factors.

Case Study Analysis

Jangho Choi, Oregon State University, assisted with these case studies.

The case studies pursued here include investigations that have targeted pond yield and profitability, water quantity and quality, native species development, and human health and safety. See Table 11 for the case-study titles and their topic areas. Those selected focus more on research than training, although some have training and outreach components. The two principal research methods used in AquaFish CRSP -- experiments and statistical surveys -- are both covered. Approximately 70% of AquaFish research has involved experimental methods and the remaining 30% survey methods.

Each case study was structured around the same set of questions, summarized in Table 12. To depict the study's setting, we examined its location and institutional background and the resources at the researchers' disposal. We next characterized the investigators' study strategy, including the research steps they planned to take, their plans for coordinating their work, and the types of professional and non-professional collaborators involved. We sought then to depict some of the dialectics of the research process, and the sequence of problems encountered and overcome. Several of the main research results and likely impacts are then summarized.

Information for these case studies was obtained from three sources: (i) the investigation proposals and quarterly, annual, and final reports; (ii) a questionnaire administered to the lead investigator; and (iii) in

some cases, email contacts with the lead investigator. The purpose of the questionnaire and email contacts was to capture aspects of the study that typically are not recorded in formal publications. We were in a few cases able to circulate case-study drafts to the lead investigators and lead host-country and U.S. principal investigators for accuracy checks. We will continue to consult with all the research leaders to improve the versions included in the present report.

The seven case studies, one from AquaFish's Phase I (2007 – 2009), five from its Phase II (2009 – 2011), and one from both the 2007 – 2009 and 2009 – 2011 phase, are provided in Appendix D.

REFERENCES

Adams, and Griliches. 1996. "Research Productivity in a System of Universities." NBER Working Paper No. 5833.

Belsley, D.A., E. Kuh, and R.E. Welsh. 1980. Regression Diagnostics: Identifying Influential Data and Sources of Collinearity. New York: John Wiley, 1980.

Buccola, S.T., D. Ervin, and H. Yang. "Research Choice and Finance in University Bioscience." *Southern Economic Journal* 75 (April 2009): 1238 - 1255

Carlin, B.P., and T.A. Louis. 1996. *Bayes and Empirical Bayes Methods for Empirical Analysis*. London: Chapman and Hall.

CGIAR Science Council. 2009. *Defining and Refining Good Practice in Ex-Post Impact Assessment – Synthesis Report.* CGIAR Science Council Secretariat: Rome, Italy.

Charnes, A., C. Cooper, and E. Rhodes, *Measuring the Efficiency of Decision Making Units*, European Journal of Operational Research, 1978, 2:429-444.

Färe, R., and S. Grosskopf. 2010. "*Directional Distance Functions and Slacks-Based Measures of Efficiency*." *European Journal of Operational Research* 200 (1): 320-322.

Groot, T., and T. Garcia-Valderrama. 2006. "Research Quality and Efficiency: An Analysis of Assessments and Management Issues in Dutch Economics and Business Research Programs." *Research Policy* 35: 1362 - 1376.

Heston, A., R. Summers, and B. Aten. 2011. *Penn World Table, Version 7.0.* Center for International Comparisons of Production, Income, and Prices, University of Pennsylvania.

Robert, C.P. 2001. The Bayesian Choice, 2nd Ed. New York: Springer-Verlag.

Press, S.J. 1989. Bayesian Statistics: Principles, Models, and Applications. New York: John Wiley and Sons.

Schimmelpfennig, D. E., and G.W. Norton. 2003. "What is the Value of Agricultural Economics Research?" *American Journal of Agricultural Economics*. 85: 81 – 94.

Stael von Holstein, C.A. 1970. *Assessment and Evaluation of Subjective Probability Distributions.* The Economics Research Institute, Stockholm School of Economics.

Tone, K. 2001. "A Slacks-Based Measure of Efficiency in Data Envelopment Analysis." *European Journal of Operational Research* 130 (3): 498 – 509.

Winkler, R.L. 1972. *An Introduction to Bayesian Inference and Decision*. New York: Holt, Rinehart, Winston.

Xia, Y., and S.T. Buccola. 2005. "University Life Science Programs and Agricultural Biotechnology." *American Journal of Agricultural Economics* 87: 229 – 243.

Table 1. Example of a Comparison of Prior and Posterior Outcome Density Function, by Research Outcome for a Given Investigation and Research Treatment [a]

Research Outcome	Possible Outcome Level		Prior probability	Posterior mean	Posterior standard deviation
Fish harvest yield (kg/ha)	Low	7000	0.10		
	Median	8000	0.60	8255.06	1438.05
	High	9000	0.30		
Dissolved oxygen (mg/L)	Low	6	0.20		
	Median	7	0.70	6.74	1.05
	High	8	0.10		
Total suspended solids (mg/L)	Low	20	0.10		
	Median	30	0.70	47.12	12.33
	High	40	0.20		

[a] Example is drawn from Investigation #07MNE04UM, "Assessing Effectiveness of Current Waste Management Practices for Intensive Freshwater and Marine Pond Aquaculture in China."

Table 2. Types of Research Inputs.

Money Expenditures ($\mathbf{E}_{hi}$)

(a) AquaFish-covered expenditure
(b) U.S. institution expenditure (cost share)
(c) host-country institution expenditure (cost share)

Human Capital ($\mathbf{H}_{i}$)

(a) research team total FTE
(b) research team size
(c) research team mean age
(d) research team mean education
(e) collaborator average education
VI.

Research Problem Type ($\mathbf{T}_{ijk}$)

(a) analytical approach (experiment versus statistical survey)
(b) investigation sample size
(c) research outcome dimension (production, marketing, species development, water quality)
(d) AquaFish Topic Area category (production, human health, technology adoption, marketing, environment)
VII.

Public Infrastructure ($\mathbf{I}_{i}$)

(a) Mean roundtrip distance to study sites
(b) Road type
(c) Transportation type
(d) Region of world (representing climate, culture, or other geographic conditions)
(e) Wage rates indexes as representative of factor prices

Table 3. AquaFish Topic-Area Categories Examined in Analysis of Knowledge Production

Topic Area	AquaFish Code
Category I: Fish Production	
Production System Design & Best Management Alternatives	BMA
Sustainable Feed Technology	SFT
Indigenous Species Development	IND
Quality Seedstock Development	QSD
Category II: Human Health	
Human Health Impacts of Aquaculture	HHI
Food Safety and Value-Added Product Development	FSV
Category III: Technology Adoption	
Technology Adoption and Policy Development	TAP
Category IV: Fish Marketing	
Marketing, Economic Risk Assessment, and Trade	MER
Category V: Environment	
Watershed and Integrated Coastal Zone Management	WIZ
Mitigating Negative Environmental Impacts	MNE

Note: Topic Areas and codes in this table are official AquaFish designations. Categories shown are our own and are for purposes of the present Investigation only.

Table 4. Definitions of Explanatory Variables Used in Statistical Analysis, 2009 – 2011 AquaFish Research Investigations.

Money Expenditure (E_{hi})	
AquaFish expenditure	Investigation expenditure financed by AquaFish
Host-Country cost share	Investigation expenditure financed by Host-Country Institution
US Institution cost share	Investigation expenditure financed by U.S. Principal Investigator's Institution
Human Capital (H_i):	
Team Size	Number of researchers on investigation team
Team Mean Education	Mean number of school/university years of research team member
Team FTE	Total annual full-time-equivalent labor of research team
Team Average Age	Mean age of research team member
Collaborator Hours Worked	Total working hours of non-team individuals helping with the investigation
Collaborator Education	Mean number of school or university years per collaborator
Other	
Analytical Approach	1 if approach is controlled experiment; 0 if statistical survey
Outcome Sample Size	Number of observations of experimental treatment effect or survey response
Country Wage Rate Index:	Mean wage rate in country (base = Tanzania)
Research Problem Type (T_{ijk})	
Research Outcome Dimension	
Fish Production	1 if outcome relates to fish production; 0 otherwise
Fish Marketing	1 if outcome relates to fish marketing; 0 otherwise
Species Development	1 if outcome relates to species development; 0 otherwise
Water Quality	1 if outcome is a water quality observation; 0 otherwise
Topic-Area Category	
Fish Production	1 if Topic-Area is in fish production; 0 otherwise
Human Health	1 if Topic-Area is in human health ; 0 otherwise
Technology Adoption	1 if Topic-Area is in technology adoption; 0 otherwise
Fish Marketing	1 if Topic-Area is in fish marketing; 0 otherwise
Environment	1 if Topic-Area is environmental 0 otherwise

Public Infrastructure (I_i)

Road Type

Paved	1 if roads from station to research sites are mostly paved; 0 otherwise
Gravel	1 if roads from station to research sites are mostly gravel; 0 otherwise
Dirt	1 if roads from station to research sites are mostly dirt; 0 otherwise

Transportation

Walking	1 if mostly walking to research sites; 0 otherwise
Auto/Bus	1 if mostly taking auto or bus to research sites; 0 otherwise
Mean Distance	Total mean distance from research station to research sites

Region of World (G_i)

Asia	1 if investigation is in Asia; 0 otherwise
Africa	1 if investigation is in Africa; 0 otherwise
Latin America	1 if investigation is in Mexico, Nicaragua, or Guyana; 0 otherwise

Table 5. Summary Statistics of Sample Data, 2009 – 2011 AquaFish Research Investigations (N = 284)

Variable	Unit	Mean	Standard Deviation	Coefficient of Variation
Knowledge Generated				
Value of Sample Information (*K*)	proportion	0.145	0.162	1.12
Mean Surprise (*MAD*)	"	0.171	0.195	1.14
Accuracy (Standard Deviation)	"	0.335	0.370	1.10
Money Expenditure (E_{hi})				
AquaFish Expenditure	$/investigation	90,645	35,162	0.39
Host-Country Cost Share	$/project	82,438	15,419	0.19
US Cost Share	$/project	175,353	44,475	0.25
Human Capital (H_i)				
Team Size	no. of persons	9.93	4.5	0.45
Team Mean Education	years/person	17.1	1.0	0.06
Team *FTE*	years	3.24	1.13	0.35
Team Average Age	years/person	32.1	4.3	0.13
Collaborator Education	years/person	13.1	3.37	0.26
Collaborator Hours Worked	hours	143.23	152.57	1.07
Other				
Experiments *vs* Surveys	1 = experiment	0.69	0.46	0.67
Country Wage-Rate Index	index	0.471	0.25	0.53
Research Problem Type ($\mathbf{T}_{ijk}$)				
Research Outcome Dimension				
Fish Production	category	0.630	0.48	0.76
Fish Marketing	"	0.134	0.34	2.54
Species Development	"	0.113	0.32	2.83
Water Quality	"	0.123	0.33	2.68

Research Topic-Area Category				
Fish Production	category	0.577	0.49	0.85
Human Health	"	0		
Technology Adoption	"	0.042	0.20	4.76
Fish Marketing	"	0.158	0.36	2.28
Environment	"	0.222	0.42	1.89
Public Infrastructure (I_i)				
Road Type				
Paved	category	0.62	0.49	0.79
Gravel	"	0.14	0.34	2.43
Dirt	"	0.24	0.43	1.79
Transportation Type				
Walking	category	0.07	0.26	3.71
Auto/Bus	"	0.93	0.26	0.28
Mean Distance to Study Site	kilometers	1132.8	2067.0	1.82
Region of World (G_i)				
Asia	category	0.81	0.39	0.48
Africa	"	0.19	0.39	2.05
Latin America	"	0		

Table 6. Factors Affecting Absolute Difference Between Prior and Posterior Mean Finding (Mean Surprise), 2009 – 2011 AquaFish Research Investigations.

Research Input	Estimate	Standard Error	t-value
Intercept	-1.299	0.761	-1.71
Continuous Inputs			
Team FTE	0.121	0.068	1.78
Team Mean Education	0.397	0.266	1.49
Mean Distance to Study Site	-0.010	0.007	-1.44
Analytical Approach			
Experiments *vs* Surveys (Base: Statistical Surveys)	-0.075	0.068	-1.11
Research Outcome Dimension			
Fish Production	-0.043	0.051	-0.84
Fish Marketing	0.072	0.070	1.03
Species Development (Base: Water Quality)	-0.045	0.102	-0.44
Research Topic-Area Category			
Fish Production	-0.020	0.069	-0.30
Human Health	-0.109	0.089	-1.23
Environment (Base: Fish Marketing)	-0.030	0.080	-0.37
Region of World			
Asia (Base: Africa and Latin America)	-0.328	0.082	-4.02

Table 7. Factors Affecting Standard Deviation (Inaccuracy) of Research Findings, 2009 – 2011 AquaFish Research Investigations.

Research Input	Estimate	Standard Error	t-value
Intercept	-1.489	0.787	-1.89
Continuous Variables			
Team FTE	-0.062	0.071	-0.88
Team Mean Education	0.709	0.275	2.58
Mean Distance to Study Site	0.006	0.007	0.83
Analytical Approach			
Experiments *vs* Surveys	-0.593	0.070	-8.45
(Base: Statistical Surveys)			
Research Outcome Dimension			
Fish Production	-0.278	0.053	-5.26
Fish Marketing	-0.130	0.073	-1.79
Species Development	-0.483	0.105	-4.58
(Base: Water Quality)			
Research Topic Area Category			
Fish Production	-0.137	0.071	-1.93
Human Health	-0.501	0.092	-5.47
Environment	-0.195	0.082	-2.36
(Base: Fish Marketing)			
Region of World			
Asia	0.061	0.084	0.72
(Base: Africa and Latin America)			

Notes

Dependent variable:	Standard deviation of experimental finding or survey response
Residual standard error:	0.222
Sample size:	284
Multiple R-square:	0.649

Table 8. Decomposition of Research Inputs' Net Knowledge Effects, 2009 – 2011 AquaFish Research Investigations.

Research Input	Knowledge Contribution *via* Research Mean Surprise	Knowledge Contribution *via* Research Accuracy	Total Knowledge Contribution
Continuous Variables			
Team FTE	0.106	0	0.106
Team Mean Education	0.346	-0.072	0.274
Mean Distance to Study Site*	0.009	0	0.009
Analytical Approach			
Experiments *vs* Surveys (Base: Statistical Surveys)	0.066	0.060	0.126
Research Outcome Dimension			
Fish Production	0	0.028	0.028
Fish Marketing	0.063	0.013	0.076
Species Development (Base: Water Quality)	0	0.049	0.049
Research Topic Area Category			
Fish Production	0	0.014	0.014
Human Health	0.095	0.051	0.146
Environment (Base: Fish Marketing)	0	0.020	0.020
Region of World			
Asia (Base: Africa and Latin America)	0.286	0	0.286

Notes: Contributions in the first column are elasticities in Table 5 multiplied by mean surprise's marginal positive contribution (0.873) to scientific knowledge. Contributions in the second column are elasticities in Table 6 multiplied by standard deviation's marginal negative contribution (- 0.101) to scientific knowledge. Numbers for the continuous inputs are percentage changes induced by a one-percent change in the indicated input. Those for categorical variables are percent changes associated with switching from the base group to the group indicated. * Mean distance effects refer to a *reduction* in mean distance.

Table 9. Technical Efficiency Analysis of 2007 – 2011 AquaFish Training Investigations, Full Data Set.

Investigation	Location	AquaFish Expenditure			US Cost Share			HC Cost Share			No. of Workshops			Training Days	*DSDM*
		x_1	x_1^d	x_1^d / x_1	x_2	x_2^d	x_2^d / x_2	x_3	x_3^d	x_3^d / x_3	x_4	x_4^d	x_4^d / x_4	y	
07SFT04UA	Guyana	15389	3236	21%	154861	18241	12%	237110	7510	3%	3	0	15%	62	3.78
07BMA03UA	Mexico	14600	3914	27%	154861	22071	14%	237110	9090	4%	1	1	55%	75	3.72
07MER02PU	Ghana & Kenya	147828	2868	2%	153884	16184	11%	93853	6666	7%	2	0	20%	55	3.70
07TAP01UC	Cambodia	98898	5271	5%	167036	29716	18%	128100	12240	10%	3	1	25%	101	3.47
07WIZ01PU	Ghana	122912	5842	5%	153884	32954	21%	93853	13575	14%	1	1	82%	112	3.18
09BMA06UM	Thailand	65829	4854	7%	192500	27360	14%	89543	11272	13%	1	1	68%	93	3.04
07QSD02PU	Western Kenya	84187	3132	4%	153884	17654	11%	93853	7272	8%	1	0	44%	60	3.04
09FSV01UC	Cambodia	62281	2557	4%	158408	14418	9%	77185	5939	8%	1	0	36%	49	2.75
07MER03PU	Tanzania	37000	1566	4%	153884	8824	6%	93853	3636	4%	1	0	22%	30	2.71
07MNE07UM	China	30000	2505	8%	140780	14120	10%	112300	5820	5%	1	0	35%	48	2.61
09SFT02PU	Kenya	78406	7307	9%	187883	41193	22%	55185	16968	31%	3	1	34%	140	2.56
09IND06PU	Ghana	78263	11013	14%	187883	62083	33%	55185	25574	46%	2	2	77%	211	2.23
07BMA04UH	Mexico	32291	1461	5%	104849	8238	8%	63287	3394	5%	2	0	10%	28	1.87
09TAP03UC	Cambodia	61945	14509	23%	158408	81794	52%	77185	33694	44%	8	2	25%	278	1.68
09FSV03UC	Cambodia & Vietnam	100000	5480	5%	35369	30893	87%	77185	12726	16%	2	1	38%	105	1.63
09QSD05PU	Ghana	100000	7985	8%	69936	45016	64%	55185	18544	34%	2	1	56%	153	1.54
07HHI04UH	Mexico	37000	6733	18%	104849	37955	36%	63287	15635	25%	1	1	94%	129	1.45
07MNE02NC	Indonesia & Philippines	40805	23486	58%	155902	132400	85%	60657	54541	90%	7	3	47%	450	0.47
07TAP02NC	Philippines	19438	19438	100%	155902	155902	100%	60657	60657	100%	1	1	100%	168	0
09BMA02AU	Uganda	50000	50000	100%	281865	281865	100%	116112	116112	100%	7	7	100%	958	0
07HHI03UH	Mexico	40000	40000	100%	104849	104849	100%	63287	63287	100%	2	2	100%	319	0
09HHI02UH	Mexico & Nicaragua	20000	20000	100%	158678	158678	100%	101774	101774	100%	2	2	100%	314	0
	Mean	**60776**	**11053**	**29%**	**149562**	**61019**	**42%**	**95716**	**27542**	**35%**	**2**	**1**	**54%**	**179**	

Table 10. Technical Efficiency Analysis of 2007 – 2011 AquaFish Training Investigations, Reduced DataSet.

Investigation	**Location**	**AquaFish Expenditure**			**US Cost Share**			**HC Cost Share**			**No. of Workshops**			**Training Days**	***DSDM***
		x_1	x_1^d	x_1^d / x_1	x_2	x_2^d	x_2^d / x_2	x_3	x_3^d	x_3^d / x_3	x_4	x_4^d	x_4^d / x_4	y	
07SFT04UA	Guyana	15389	5622	37%	154861	21481	14%	237110	8360	4%	3	1	32%	62	3.72
07BMA03UA	Mexico	14600	10990	75%	154861	35941	23%	237110	17710	7%	1	1	100%	75	3.42
07TAP01UC	Cambodia	98898	9158	9%	167036	34996	21%	128100	13610	11%	3	2	52%	101	3.36
09FSV01UC	Cambodia	62281	4443	7%	158408	16978	11%	77185	6605	9%	1	1	76%	49	2.70
07MNE07UM	China	30000	4353	15%	140780	16630	12%	112300	6470	6%	1	1	75%	48	2.56
09SFT02PU	Kenya	78406	12695	16%	187883	48503	26%	55185	18871	34%	3	2	73%	140	2.41
09BMA06UM	Thailand	65829	19660	30%	192500	58910	31%	89543	32901	37%	1	1	100%	93	2.36
07WIZ01PU	Ghana	122912	28812	23%	153884	83157	54%	93853	48938	52%	1	1	100%	112	2.10
07BMA04UH	Mexico	32291	2539	8%	104849	9701	9%	63287	3774	6%	2	0	22%	28	1.84
09TAP03UC	Cambodia	61945	25208	41%	158408	96313	61%	77185	37473	49%	8	4	54%	278	1.39
07HHI04UH	Mexico	37000	37000	100%	104849	104849	100%	63287	63287	100%	1	1	100%	129	0
07MNE02NC	Indonesia & Philippines	40805	40805	100%	155902	155902	100%	60657	60657	100%	7	7	100%	450	0
09QSD05PU	Ghana	100000	100000	100%	69936	69936	100%	55185	55185	100%	2	2	100%	153	0
09FSV03UC	Cambodia & Vietnam	100000	100000	100%	35369	35369	100%	77185	77185	100%	2	2	100%	105	0
09IND06PU	Ghana	78263	78263	100%	187883	187883	100%	55185	55185	100%	2	2	100%	211	0
	Mean	**62575**	**31970**	**51%**	**141827**	**65103**	**51%**	**98824**	**33747**	**48%**	**3**	**2**	**79%**	**136**	

Table 11. Investigations Examined in a Case Study

U.S. University	Investigation #	Investigation Title	Principal Host-Country Investigator
University of Michigan	09WIZ03UM	Evaluating efficiency of nutrient and solid waste retention of traditional and improved cages for fish culture in deep lake waters in China (Mitigating negative environmental impact/Study)	Zexia Gao
Auburn University	09WIZ01AU	Effects of Watershed-Water Quality-Aquaculture Interactions On Quantity and Quality of Water from Small Catchments in South Africa and Uganda	Khalid Salie
University of Arizona	09IND05UA	Consolidation of native species aquaculture in Southeastern Mexico: Continuation of a Selective Breeding Program for Native Cichlids and snook reproduction in captivity	Wilfrido Contreras
	07IND01UA	Development of Snook (*Centropomus spp*) Seed Production Technology for Application in Aquaculture and Restocking of Over-fished Populations (Tabasco)	
University of Hawaii	09HHI04UH	Co-management and bivalve sanitation for black cockles (Anadara spp.) in Nicaragua	Erick Sandoval
University of Connecticut	09SFT01UC	Alternative Feeds for Freshwater Aquaculture Species.	Tran Thi Thanh Hien
Purdue University	07WIZ01PU	Characterization of Pond Effluents and Biological and Physiochemical Assessment of Receiving Waters in Ghana	Emmanuel Frimpong Steve Amisah
North Carolina State University	09SFT04NC	Feeding and Feed Formulation Strategies to Reduce Production Costs of Tilapia Culture	Remedios Bolivar

Table 12. General Format of Case Study

I. Problem

(i) What difficulty are researchers trying to address?
(ii) How will they try to address it?

II. Study Setting

(i) Where is the study conducted?
(ii) Who conducts it?
(iii) What study resources will researchers have?
(iv) What is the geographic setting or other relevant environment of study?

III. Study Strategy or Plan

(i) How in general do researchers solve the problem?
(ii) What steps do they to take?
(iii) How will researchers coordinate their work with one another?
(iv) What kinds of non-professional collaborators (e.g. farmers) are involved, and how?
(v) What kinds of professional collaborators (e.g. university administrators, AquaFish administrators) are involved, and how?

IV. Problem Solution Process

Phase One: achievement and problems
Phase Two: achievement and problems
Phase Three: achievement and termination

V. Results

VI. Likely Impacts

Appendix A. Outputs Questionnaire for Controlled Experiments

CRSP PHASE: [ ] 2007-09 [ ] 2009-11
INVESTIGATION TITLE OR NUMBER: _____
STUDY #:_____

	e.g., feed composition, frequency, amount	e.g., soils, vegetation	e.g., fingerling growth rate	e.g. from literature review, professional experience		from controlled trials		**Notes** (include sample size)
Year & Treatment #	**Management Treatment**	**Facilities/Equipment**	**Outcome Target** (indicate unit)	**Prior Probability Distribution**		**Experimental Results**		
				Outcome Level	**Probability of Occurrence (%)**	**Mean** (indicate unit)	**Standard Deviation**	
				High _____ Medium _____ Low _____	_____ _____ _____ 100%	_____	_____	
				High _____ Medium _____ Low _____	_____ _____ _____ 100%	_____	_____	

Appendix B. Outputs Questionnaire for Survey Studies

CRSP PHASE: [ ] 2007-09 [ ] 2009-11
INVESTIGATION TITLE OR NUMBER: _____
STUDY #:_____

	e.g. demand seasonality, estuary quality, fish population	e.g., from survey pre-test or rapid reconnaissance		formal survey statistics		
Year & Dimension #	**Dimension of Problem Examined**	**Prior Probability Distribution**		**Survey Results**		**Notes** (include sample size)
		Dimension Level (indicate unit)	**Probability of Occurrence (%)**	**Mean (indicate unit)**	**Standard Deviation**	
		High_____ Medium_____ Low_____	_____ _____ _____	_____	_____	
		High_____ Medium_____ Low_____	_____ _____ _____	_____	_____	
		High_____ Medium_____ Low_____	_____ _____ _____	_____	_____	

Appendix C. Inputs Questionnaire

Please first complete the following information:

Personnel information					
Name	Rank	Age	Highest Degree	FTE per year devoted to this investigation	Other Information

- ☐ Controlled experiment
- ☐ Statistical survey
- ☐ Outreach or training

A second way to characterize an investigation is by its *geographical situation*:

- ❖ **On the premises of a university or research station (**so there is no need for the investigators to travel away from their place of work**).**
- ❖ At a *single site* located some distance away from the university or research station (for example, at a farm or very closely situated group of farms).
- ❖ At a *collection of sites* located some distance away from the university or research station (for example, fish processors interviewed at a variety of sites across the country).

Please check which of the above three categories *best* describes the present investigation. (You may choose more than one type, for example if your work is substantially conducted both at the university and away from it.)

Geographical situation:

☐ On the premises of your university or research station

☐ At a single site some distance from university or research station

☐ At multiple sites some distance from university or research station.

Single Off-Station Site:

If you checked **box #2** on page 4, please answer the following:

1. Distance of the single off-station site from your research station:

   _____

2. Transportation method to the site:

   ☐ Walking ☐ Bicycle ☐ Auto ☐ Bus ☐ Other _____

3. Road conditions to the site:

   ☐ Paved ☐ Gravel ☐Dirt ☐ Other_____

4. Do you have access to a mobile phone at the site? ☐ Yes ☐ No

   Explain:

   _____

Multiple off-station sites:

If you instead checked **box #3** on page 4, please answer the following:

1. What kinds of multiple research sites are you referring to? (E.g., fish exporters' offices or fish farms involved in research or visited as part of workshop)

   _____

2. Number of off-station research locations: _____

3. Total mileage required for you to visit all these off-station locations (if you take the most efficient route to visit all of them): _____

4. Transportation method to these multiple sites:

   ☐ Walking ☐ Bicycle ☐ Auto ☐ Bus ☐ Other _____

5. Road conditions to the average site:

   ☐Paved ☐ Gravel ☐Dirt ☐Other_____

6. Do you have access to a mobile phone at these sites? ☐ Yes ☐ No

Explain:

A **third way** to characterize an investigation ***by the non-AquaFish collaborator(s)*** **with whom you worked or are working:**

- **Without collaborators:** Your work is conducted only with AquaFish CRSP personnel (including paid laborers)
- **With collaborators:** Your work is conducted in collaboration with other people (for example, farmers, exports brokers, fish processors)

Please check one of the following:

Use of collaborators:

☐ Without collaborator(s)

☐ With collaborator(s)

- If your investigation is conducted with collaborators (that is, if you checked **box 2** on page 7):

1. Brief description of work the collaborator(s) perform or performed

_____

2. Number of collaborators: _____

3.Collaborators' average educational level:

_____

4.Average number of hours per month the collaborator(s) worked with the investigation:

_____

Other conditions affecting you ability to carry out your investigation:

Please describe any other factors affecting the cost of your research (e.g., rural infrastructure)

Appendix D. Case Studies
Alternative Feeds for Freshwater Aquaculture Species

Case Study of AquaFish Investigation 09SFT01UC

Jangho Choi, Lin Qin, and Steven Buccola

Principal Investigator:

Tran Thi Thanh Hien, Vice-Dean, College of Aquaculture and Fisheries, Cantho University, Vietnam.

Introduction

Vietnam has experienced rapid growth in aquaculture and Cambodia has the potential to do the same. Feeding cost consumes about 70% of snakehead culture cost. Many small farmers continue to draw their feeds from "trash fish" caught in fresh or marine water fisheries, while larger farms use pelleted feed from commercial feed mills. On-farm feed formulation have not been widely used in Vietnamese or Cambodian aquacultural systems.

The present investigation examined the possibilities of alternative feeds for snakehead production in Vietnam, using locally available plant materials to build a long-term sustainable aquaculture system. AquaFish researchers and scientists from the University of Rhode Island and Cantho University worked together to carry out a series of formulated-feed experiments and on-farm snakehead culture trials. The study included an economic analysis of fish-meal costs, a productivity comparisons of plant-based proteins and trash fish, and an analysis of the risks of an unavailability of trash fish in the decades ahead.

Links to Earlier Research

This investigation was the second phase of a study began in 2007. During the study's 2007 – 2009 phase, the team had determined the species composition, size, and chemical composition of the main freshwater trash ("low-value") fish species used as feed in finfish aquaculture in Vietnam's Mekong Delta. They also had developed weaning methods allowing small, hatchery-reared snakehead to become quickly adapted to pelleted diets. They had, for example, assessed *Channa striata* snakehead survival and growth prospects in the presence of selected diet types. They showed that a feed consisting of fish, soybean, and cassava meal – which can be cheaply and locally produced and formulated – can replace up to 30% of the more expensive rice-bran content with no decline in snakehead survival or growth.

Experimental Design

Formulated-feed experiments were conducted in the wet laboratory and hapas at the College of Aquaculture and Fisheries of Cantho University, and on-farm trials carried out in An Giang and Dong Thap Provinces. Research consisted of eight interlocking studies, each with multiple experiments.

Study 1 evaluated the chemical composition of marine and fresh-water trash fish to determine their relative values as a snakehead feed ingredient. Samples were collected from three different sites and analysis conducted of their chemical composition (protein, lipid, moisture and mineral) and TVB-N (Total Volatile Base Nitrogen).

Study 2 was comprised of pilot trials for weaning snakehead larvae onto formulated feeds. It consisted of trials for snakehead *(Channa micropeltes)* larvae, snakehead *(Channa striata)* larvae on farms; and giant snakehead *(Channa micropeltes)* fingerlings. The experiment considered larvae ten days after hatch,

carrying out with nine treatments with three replicates per treatment. Trash-fish feed was replaced by experimental feed at 20, 30 or 40 days after hatch – at rates of 10% per day, 10% every two days, or 10% every three days – until larvae were receiving 100% of the experimental feed per day. Fish then were counted and final body weight and wet-weight gain determined. Based on these data, survival rate, daily weight gain, feed intake, feed conversion ratio, protein efficiency ratio, and economic conversion ratio were calculated. The mortality rate (number of actual mortalities removed from experiment) and cannibalism rate (initial number of fish stocked minus number of mortalities removed less survivors) were analyzed as well.

Study 3 compared *Channa striata* growth when fed a formulated rather than trash-fish feed. In pond #1, snakehead fingerlings (12-13g/fish) in three 5x10 meter hapas were fed marine trash fish for six months. In pond #2, snakehead fingerlings (12-13g/fish) in the three 3 hapas were provided a formulated feed consisting of such ingredients such as Kien Giang fish meal, defatted soybean meal, cassava meal, and dried rice-bran. All diets were made in an extruding-pellet mill at Cantho University. Trash fish was marine trash fish bought from markets and was chopped up before feeding. At the end of the experiment, the team conducted a sensory comparison of the fillet quality of the experimental and wild fish, and an analysis of the feeds' chemical compositions.

Study 4 applied the Study 3 methods to *Channa micropeltes* rather than *Channa striata.* Six hapas ($50m^2$/hapa) were placed in one pond (700 m^2) with a stocking density of 100 fingerlings/m^2. Giant snakehead fingerlings (12 - 13g/fish) in three hapas (5x10 m in size) were fed marine trash fish for 5 months, and fingerlings (12-13g/fish) in the other three hapas (5x10 m in size) fed a formulated feed.

Study 5 replaced the traditional marine trash-fish diet – then a freshwater trash-fish diet – with rice bran and with rice bran mixed with cassava meal. In each of these settings eleven separate diet treatments were used, including a formulated pellet determined during the 2007 – 2009 phase of this investigation, and others feeds involving combinations of marine and freshwater trash-fish, rice, and cassava meal. Three replicates were employed in each treatment.

In *Study 6* the research team moved to on-farm trials, in which fingerlings were fed floating commercial pellets. Pellets in the 1st, 2nd, and 3rd months contained 44% crude protein (CP), and only 40% CP afterward.

In *Study 7*, 29 farmers were interviewed in An Giang and 12 in Dong Thap about their fish-culture and fish-health management practices, about disease outbreaks in their ponds, and about profitability. Related secondary aquacultural information also was collected from newspapers, magazines, and websites.

Significant Outcomes

The laboratory experiments and on-farm trials confirmed that formulated feeds provide An Giang snakehead farmers significantly higher profit than do trash-fish feeds. Pellets formulated with local rice-bran, cassava, and freshwater trash-fish produce given weight gains in a less costly way than marine trash-fish does. Feed conversion ratios and returns on equity therefore are higher.

Specifically, snakehead fingerlings can be weaned at the rate of 10% per day from a trash-fish to formulated-feed diet. Neither growth nor survival rate significantly changed from 100% trash-fish use. Among giant snakehead, trash-fish-based fingerling feeds did bring significantly higher daily weight gain than did formulated feeds. However, they brought no significant advantage in survival rate. Quality analysis showed that on day of capture, trash fish from fresh water satisfied a higher freshness standard than did trash fish from marine sources. And freshwater trash fish provided more effective feeds for *Channa striata* than did those from marine waters.

Improved Cages for Fish Culture in Deep-Water Lakes

Case Study of AquaFish Investigation 09WIZ03UM

Lin Qin, Jangho Choi, and Steven Buccola

Principal Investigator:

Liping Liu, Associate Professor, College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China

Introduction

Cage culture is often used in Asia's lakes, reservoirs, and slower-flowing rivers to improve both fish production and water quality, allowing a more intensive feeding strategy and greater carrying capacity. However, it also can be environmentally problematic because cages allow solid and liquid wastes to disperse into the receiving waters, leading to eutrophication, pollution, and habitat degradation.

VII.

The purpose of the present investigation was to compare the fish production, water quality, and carrying capacity performance of a traditional and an ecologically improved freshwater aquaculture cage. Specifically, the investigation assessed the impacts of the improved cages designed to reduce waste loadings into Longtan Reservoir in southern Guizhou Province. The improved cage in the present study was designed by the Tongwei Group of China (Figure 1). It consists of a collecting cone and tube built at the cage bottom to collect solid and liquid waste, plus a surrounding cage (or outer cage) to retain nutrients for growing additional fish species. AquaFish CRSP researchers and scientists from Shanghai Ocean University, Huazhong Agricultural University, and University of Michigan worked together to test whether the improved cage has significant advantages over the traditional one.

VIII.

Links to Earlier Research

IX.

During the AquaFish CRSP's 2007-2009 phase, this same team had assessed (07MNE04UM) the effectiveness of waste management practices for intensive freshwater and marine pond aquaculture in China. In particular, they had evaluated alternative management strategies employed by small-scale farmers in Hubei and Hainan provinces to reduce effluent and solid waste pollution in intensive carp, tilapia, and shrimp pond culture. They identified, in that process, several important management improvements but had excluded deep-water cage-culture methods. Both investigations have pointed to ways of boosting fish production and reducing aquatic pollution, but with the use of different methods.

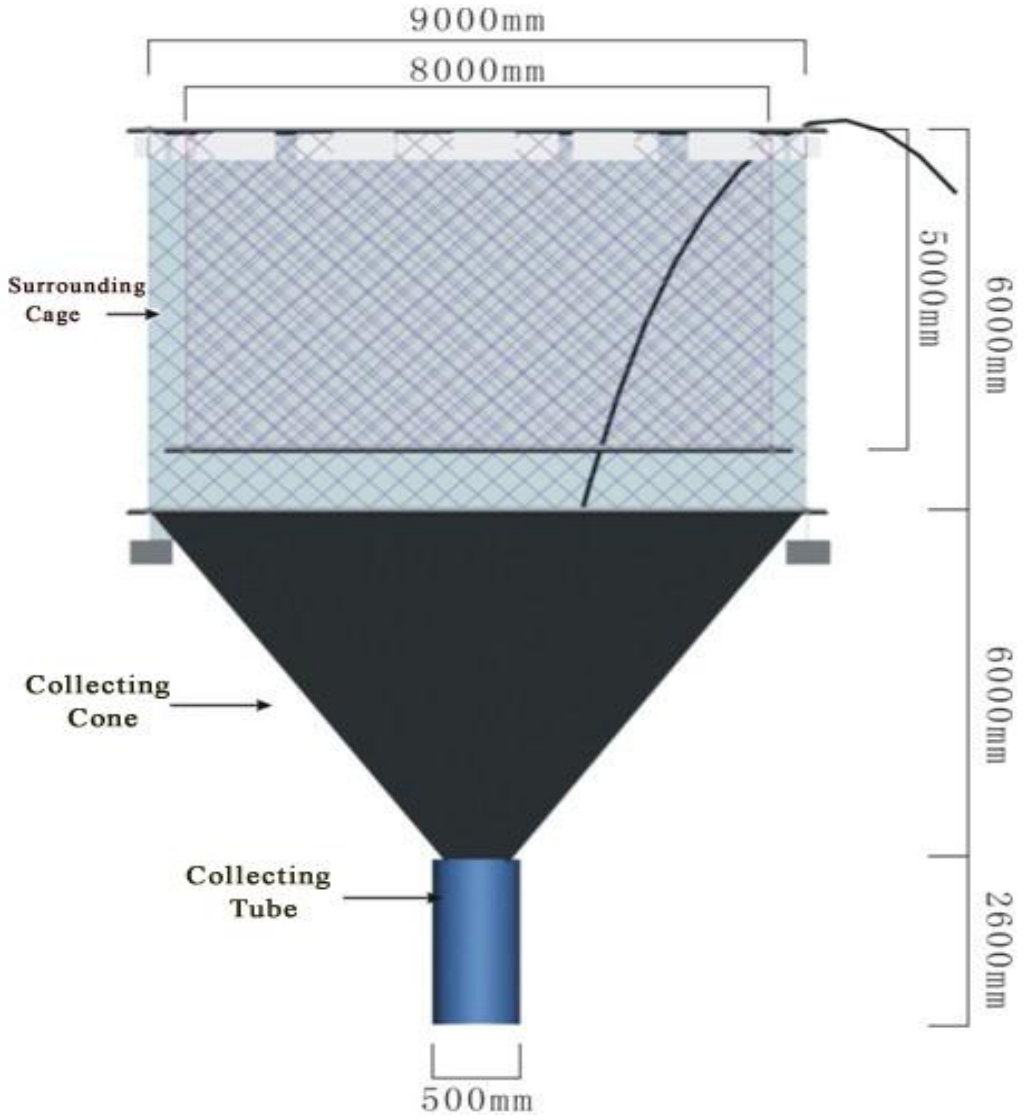


Figure 1. Diagram of the Tongwei ecological cages with sediment collection from the bottom and a surrounding cage for nutrient retention.

Experimental Design

The experiment began in May 2010. Ten cages were used in the experiment, including three modified-a cages containing an outer cage and no sediment trap, three modified-b cages containing both the outer cage and sediment trap, three traditional cages, and one control cage. Modified cages were 12x12 m in surface area and 6m deep, not including the sediment collector below. Traditional cages were 5x5 m in surface area and 5 m deep, and the control cage 3x3 m in surface area and 3 m deep. Reservoir water was used for hydropower and thus had a measureable flow.

Fish stocking density was 160 Channel catfish per square meter, or a total of 16,000 fish per cage in the modified cages, while there were 4,000 catfish in traditional cages. In the ecological cage's main-culture or inner apparatus, the stocking rate was similar. In its surrounding apparatus, it was combined with seven bighead carp (500g each), three silver carp (200g), and six tench (50g) per square meter.

Growout duration was one year. Feeding treatments differed between the inner and surrounding cages. Fish in traditional cages and the inner apparatus of the ecological cages were fed with pellet feeds at manufacturer-commended levels, while no feed or other materials were provided to the outer cages. Water quality was monitored through certain routine measurements and other, less frequent ones. The former, performed weekly, included water DO, Ph, conductivity, and temperature, drawn from a data sonde. These measures were taken both inside the cages and in the exterior water. Measures in the cages were drawn in three locations: at the first cage in the upstream group, at the center of the cage cluster, and at the last cage in the downstream group.

The more detailed measurements were made one month, six months, and twelve months after stocking. They included total phosphorus, total nitrogen, NO3, NO2, TAN (total ammonia nitrogen), TVS (total volatile solids), TSS (total suspended solids), and phytoplankton and zooplankton samples. Fish size and sedimentation rate were also measured monthly.
The AquaFish researchers expected the ecological cage-culture system to boost fish production, improve the nutrient dynamics, and reduce the pollution burden considerably in these deep waters. This expectation was tested by comparing data from four cage-culture systems using standard Analysis of Variance.

Unexpected Difficulties

The AquaFish researchers encountered several unexpected difficulties in the process of this investigation. The first came when the automatic equipment for feces collection in the ecological cage culture system became unstable during the experiment. Instead of using the automatic equipment for feces collection, they had to collect it by hand. The other arose from the difficulty of using fishing nets to sample the Channel catfish in the inner cages. The Channel catfish fins were very strong, easily destroying the fishing net. It was found much easier to draw the samples by traditional fishing methods.

In addition, fish in outer cages retained less than 1% of total phosphorus input from cages. This retention was not enough to influence nutrient concentrations between different cage types, and raised questions about the availability to filter-feeding fish of waste nutrients from intensively fed cages.

The last but not least important difficulty was that constraints in sampling ability limited the conclusions that could be drawn from the results. The remote location of the facility made sampling infrequent. And the experiment took place at a commercial facility, so how the proximity of the experiment to other cages may have confounded the results was unknown.

Communication

In addition to the collaboration among the three universities, Tongwei Company's specialists provided a substantial amount of advice on the use of the improved cages. This was a win-win situation for both sides because Tongwei benefitted from AquaFish's rigorous comparison of the traditional and Tongwei-improved cage in fish production and water pollution. Tongwei also had received government funding in its cage design work.

Because the study reservoir is a two-hour drive from the Huazhong Agricultural University campus that served as the study headquarters, local farmers were engaged to help feed the fish and collect the water samples. To better communicate with these farmers, a translator was engaged to speak the local language.

Significant Outcome

The most significant finding of this investigation was that fish production in cage-culture systems can be substantially improved by using feces collection equipment. In particular, catfish growth rates were lower in traditional than in modified cages (figure 2), giving lower production. Feed conversion ratios ranged from 1.37 to 2.61 but were not statistically differ among traditional and other treatments.

Unexpectedly, water quality did not significantly differ between traditional or experimental cages. The traditional ones held a greater number of species than the improved cages did, and all cages held more species than did the reservoir samples. Biomass of phytoplankton was fairly similar across cage types.

Economic return appeared to be greatest when culturing bighead carp and tilapia in the surrounding apparatus. The net income ratio of modified cages a and b were 74.3% and 73.8%, respectively. Cost of the waste collection device accounted for only 0.6% of the total cost. The revenue of channel catfish in modified cages a and b accounted for 96.8% and 96.4% of the total revenue, respectively. Thus, revenue from channel catfish contributed most to total revenue, while revenue from both types of modified cages was small.

Future Study

The team suggests examining the application of fish feces from the ecological cage culture system to vegetable production. In the outer cages, water quality might be further improved by culturing float grass or by using bio-membranes.

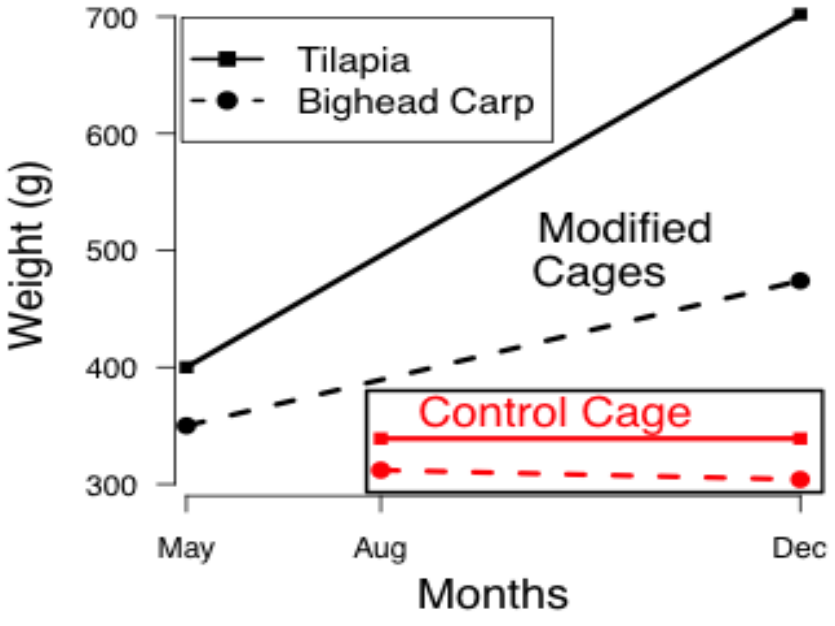


Figure 2. Average weight of carp and tilapia in modified and control cages at stocking and at harvest.

Pond Effluents and their Impacts on Receiving Waters in Ghana

Case Study of AquaFish Investigation 07WIZ01PU

Lin Qin, Jangho Choi, and Steven Buccola

Principal Investigator:

Emmanuel A. Frimpong, Research Scientist, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute & State University, Virginia, the United States.

Introduction

Regulation of water pollution in West African fish farming is hampered by inadequate biological, chemical, and managerial data on pond effluents. In the absence of that information, regulatory agencies may either exaggerate or understate aquaculture's pollutive effects, leading either to over-regulation and hence undue industry restrictions or to controls that inadequately protect the environment. There has, as a result, been increased demand for effective characterization of pond effluents and assessment of their effects on aquaculture's receiving waters.

The present study, conducted by Purdue University in collaboration with Virginia Tech and Kwame Nkrumah University of Science & Technology, sought to estimate the potential and actual impacts of pond effluents on receiving-stream quality in the Ashanti and Brong Ahafo regions of Ghana. The hope is eventually to extend the study to the entire Ghanaian aquatic ecosystem. The Ashanti and Brong Ahafo regions were chosen for their concentrated aquacultural activity, providing the variety of aquacultural system and receiving-water characteristics that would allow generalizations to the rest of Southern Ghana and similar systems in other sub-Saharan African countries.

Study Strategy

A combination of questionnaire survey, field sampling, and laboratory analyses of pond water, receiving and reference stream water, and fish and benthic macro-invertebrate sampling were used to generate the data. Six pond types were examined, and the water-quality impact of each pond type was analyzed. Ponds, receiving waters, and reference streams were first examined for macro-invertebrate and fish populations and for physicochemical and microbial content. Cluster analysis was used with the survey data to identify possible pond and effluent types. Relationships between management practices and downstream physicochemical and microbial levels were then assessed, and environmental best-management practices drawn up and disseminated through a workshop.

Unexpected Problems

X.

Two unexpected technical problems occurred during the study. The first was unpredictably high rainfall and flooding during the field sampling, requiring stream re-sampling at some stations. The second was that, because collaborators' fish ponds were widely dispersed and drained at odd intervals, so researchers were unable to observe actual effluent contents. They had instead to resort to computing potential effluent by sampling at various depths of the ponds themselves rather than actual effluents at the outlets.

XI.

Beside these technical problems, three non-technical difficulties presented themselves as well. Bureaucratic procedures delayed funds for fieldwork, so field sampling schedule had to be reworked to

begin with tasks not requiring immediate cash expenditure. Meanwhile, the bureaucratic process delayed laboratory services for water sampling at the collaborating university. And state agencies were unable to provide digitized geographical information about the study area, so that private arrangements had to be arranged later for it.

Communication

Earthen pond fish farmers, offering their farms and streams for sampling, were the main collaborators in this study. Regional heads of the Ghana Fisheries Commission, and the Ashanti Regional Director of the Ghana Statistical Service, helped throughout the study. Regional fisheries directors provided lists of fish farmers, and introduced them to the AquaFish researchers. Ghana Statistical Services provided socioeconomic information about study regions.

The fish farmers themselves appeared to be more interested than anyone else in the pond effluent examination. Not only do they want to understand why the mortality rates have been high in some of their ponds while the fish in other ponds flourished, but also they hope the water-quality measures the study will identify will help them avoid stringent state regulation. Fisheries Commission directors or their representatives used the opportunity of the study to visit the collaborating pond farms in their regions, although official and extension visits were constrained due to transportation and other problems.

A random sub-sample of at least five farms was selected from each stratum in the two regions. The investigators obtained the cooperation of local fish farmers by explaining research objectives and strategies to them in advance of the study.

Significant Outcomes

From the survey and statistical analysis, researchers concluded there was no evidence that fish farming was creating any significant water quality degradation in receiving streams, or any adverse effect on fish and benthic macro-invertebrates. This is remarkable because water qualities in Ashanti and Brong Ahafo ponds are generally quite different from those in receiving streams, which usually contain more natural nutrients, solids, and organic waste-water content than ponds do.

Despite this good news, the pollution situation in the Ghanaian fish-farm industry could worsen as the industry intensifies. The key question will be how effluents are managed, including the frequency and volume of pond-water releases and under what conditions effluents are handled before reaching the receiving waters.

This study of Ghanaian fish-farm effluent has provided government regulators with baseline information about pond aquaculture in Ghana. More importantly, however, it has provided Best Management Practices (BMPs) recommendations for fish farmers. The majority of Ghanaian fish farms actually have environmental BMPs already in place. They include water reuse mechanisms, vegetated ditches or canals, settling basins, drainage into natural wetlands, and top-release of pond water. Continuing to implement such broadly focused environmental BMPs my reduce the need for aquacultural effluent regulations in Ghana for the foreseeable future.

AquaFish scientists say a better understanding of the economic, social, and environmental impacts of adopting BMP or other practices on Ghanaian fish farms could be achieved through a systematic classification of fish farming areas, including a comprehensive spatial database and a characterization of fish pond effluent systems.

Development of Snook Seed Production Technology in Mexico

Case Study of AquaFish Investigation 07IND01UA and 09IND05UA

Jangho Choi, Lin Qin, and Steven Buccola

Principal Investigator:

Wilfrido M. Contreras-Sánchez, Professor, Laboratorio de Acuicultura Tropical,
División Académica de Ciencias Biológicas Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México

Introduction

Snook fish stocks have gradually declined in southeastern Mexico on account of the fish's great market popularity. As Mexican incomes rise, snook consumption rises also, depleting stocks in many areas. Alternatives – especially through artificial reproduction – to capturing wild snook therefore are being sought, not only for meeting market requirements but for protecting wild fisheries. Researchers from the University of Arizona and Universidad Juárez Autónoma de Tabasco have conducted a series of experiments to deal with the problems encountered when snook breeders are held in captivity.

In the first of the investigations (07IND01UA), the team developed techniques for snook seed production, including injecting or implanting wild broodstock with alternative concentrations of GnRHa (a hormone analogue that intervenes in the reproductive system). The team held an international workshop on snook biology and culture in July 2009.

In the second investigation (09IND05UA), they tested the possibility of artificial reproduction in Common, Fat, and Mexican Snook. They also looked at broodstock feeding programs, in particular the expression of and digestive-enzyme genes in snook broodfish.

XII.

Experimental design

XIII.

Major snook species are the Common Snook (*Centropomus undecimalis*) and Fat Snook (*Centropomus parallelus*). Fat Snook wild broodstock samples were collected during spawning season in coastal areas near the experiment facility, which had been established in earlier collaborations with USAID (CRSP-UJAT-Cooperativa Pesquera San Ramón). Samples were obtained from the induced spawning of broodstock held in captivity since 2008.

In experiment #1 of investigation 07IND01UA, 36 breeders (12 females and 24 males) were selected for experiments, one female and two males in each experiment. Recently-caught wild broodstock were employed in Fat Snook experiments, while one- to two-year-old captured and cultured broodstock were used in the Common Snook experiments. Treatments were either by injection or pellet at various GnRHa concentrations. Three hypotheses (Ho) about the techniques for good-quality snook egg production were tested:

Table 1. Three experiments conducted to examine techniques for producing good-quality snook eggs.

Experiment	Species	Treatments
Experiment 1	Ho: Injections of GnRHa will produce good-quality eggs	
Ex 1a	Wild Fat Snook	Females were injected with saline solution, 75 or 150 µg GnRHa/kg. All males were injected with 50 µg GnRHa/kg
Ex 1b	Common Snook	Not conducted (no wild fish)
Experiment 2	Ho: Implants of GnRHa will produce good-quality eggs	
Ex 2a	Fat Snook	Females were implanted with pelleted vehicle (no GnRHa), 100 or 200 µg GnRHa/fish. All males were implanted with 100 µg GnRHa pellets
Ex 2b-i	Common Snook	Same as Ex 2a
Experiment 3	Ho: Initial stocking rates of snook larvae will not affect their growth or ability to wean from live zooplankton to prepared diets	
Ex 3a	Fat Snook	Five liter culture tank
Ex 3b	Fat Snook	Ten liter culture tank

In experiments #2 and #3, treatment effectiveness was determined by the presence or absence of spawning and by egg quality level. The number of eggs produced was estimated by taking three 50 ml samples from the egg collector and counting eggs with a Petri dish. Using a dissecting microscope, fertilization was recorded after 30 minutes by blastemic observation of 100 eggs. For hatching estimation, viable eggs were incubated in a 300 L tank, using sea water similar to that employed in the spawning tanks. After 48 h of incubation, three 50 mL samples were taken from each incubation tank for larval-counting under microscope. Larval survival was determined by the same method. Egg number, egg diameter, larvae total length, fertilization rates, hatching, and survival-to-first-feeding were recorded and examined statistically in each treatment. Two batches of Fat Snook larvae were obtained by GnRHa pellet induction. Initial snook-larvae stocking densities were 20, 40, 60, 80, and 100 larvae/L and each treatment was replicated three times.

From July 8 to July 15, 2009, the AquaFish team conducted an international workshop at División Académica de Ciencias Biológicas, UJAT. The workshop theme was larval culture and grow-out of Fat Snook juveniles, and histological determination of gonadal development on Teleost fish. About 185 participants attended the workshop.

During the investigation's 2009 – 2011 phase, the team continued drawing broodstock samples and testing alternative technologies for good- quality snook seed production. They first identified the digestive enzyme gene expression in Common and Fat Snook at various stages in their life cycle. Snook larvae and juveniles with at least 150 mg of stomach, intestine, and pancreatic tissue were collected, and samples drawn for RNA extraction and other tests.

Unexpected Difficulties

Unfortunately, the team managed to catch no wild snook with satisfactory broodstock quality in 2009. For want of broodstock, some experiment plans had to be changed. In particular, Experiment #1b was never conducted; only one female was used per treatment in experiment #2b; and only the captured female was implanted in experiment #2b-i.

Because only Fat Snook larvae were obtained from these experiments, no Common Snook larvae could be produced. Although additional experiments were conducted at the Universidad Nacional Autonoma de Mexico to acquire Common Snook larvae, insufficient larvae were obtained to run trials on the density of GnRHa pellet induction. Thus, only Fat Snook could be used in the experiment #3 density trials. Furthermore, all Fat Snook larvae had died by the 8th day of the experiment, so that experiment #3 conclusions about the effects of initial larvae stocking rates could be obtained only for the first eight days of feeding. On account also of the paucity of female samples, possibilities for statistical inference were weak.

Significant Outcomes

The AquaFish team concluded in investigation 07IND01UA that both injections and implants were effective in inducing Fat Snook spawning, finding no significant differences between these methods in the numbers of eggs produced, in fertilization, or in hatching rates.

XIV.

In investigation 09IND05UA, they determined the possibility of Common and Mexican Snook spawn and larvae production by GnRHa-induced reproduction. Snook populations thus can be maintained through hormonal stimulus, and improved feeding and handling strategies can maintain broodstock for lengthy periods. However, effective GnRHa dose varies by broodstock weight.

XV.

Although inadequate size of the sample broodstock prevented statistical tests, this AquaFish study has showed snook broodstock can readily be adapted to captivity conditions, where only hormone-treated females can spawn. Higher-dose. Fertilization percentage was 100%, higher-dose females produced more eggs, and hatching rate was 68%.

Co-Management and Bivalve Sanitation for Black Cockles in Nicaragua

Case Study of AquaFish Investigation 09HHI04UH

Lin Qin, Jangho Choi, and Steven Buccola

Principal Investigator:
Erick José Sandoval Palacios, Assistant Professor, Central American University, Managua, Nicaragua.

Introduction

Black Cockles are – along most of the Pacific coast of Latin America – an important fisheries resource and emergency food, especially among women, children, and the poor. Unfortunately, on account of imprudent management, this resource is faced nearly with extinction. Governments in some countries in turn have introduced yearly bans on their exploitation, threatening local fishers' incomes. Because most black cockles are collected from the contaminated estuary areas and eaten raw, the bivalve sanitation also has become an urgent issue for aquacultural researchers and scientists.

The present study was part of AquaFish CRSP's efforts in Human Health and Aquaculture, focusing on aquacultural sanitation and best-management practices. As a continuation of an earlier (2007 – 2009) study, it sought to examine how voluntary community ("co-management") programs might be used to effectively stabilize cockle populations and improve cockle quality in heavily harvested areas. Cockle areas designated as "no-take" were compared with others in terms of cockle numbers, size, and tissue quality in the Aserradores Estuary.

Links to Earlier Research

Prior to the study, community co-management already had successfully improved average cockle sizes in no-take zones of the Aserradores Estuary, which were heavily exploited. The community voluntarily selected the zones, which could be successively expanded if found effective. As scientists began pointing to co- management's successes, the Ministry of Natural Resources (MARENA) considered giving it official sanction. But before it could be extended to other communities, long-term evidence would have to be provided of its positive effects on cockle volumes and safety.

The Center for Development and Research of Aquatic Resources (CIDEA) at Central American University (UCA), and its partners at the University of Hawaii - Hilo, had for the past decade studied black-cockle culture, management, and sanitation methods on the Nicaraguan Pacific Coast. Their purpose was to test community-based co-management programs as an alternative to the inefficient, year-by-year April-July collection bans, which had little scientific validity and place local fishers into conflict with government regulators. The AquaFish investigation was intended to supplement these efforts by examining the extent of the black-cockle population, size, and sanitations problem and what can be done to improve it.

Research Design

Beginning in January 2010, cockle populations were drawn every six months at each of twelve sampling stations, including three no-take zones, three sites 100 meters from those zones, and six sites far away from the no-take zones. No-take zones were selected by community leaders, marked with signals along the perimeters, and altered every six months. Local Fishers were employed as samplers, trained in sampling methods, and regularly visited by AquaFish staff during the sampling process. The samples were used in one-way analysis of variance (ANOVA) to test whether the cockle population differed significantly between the no-take zones and those at some distance from them.

In the meantime, CIDEA facilitated community meetings in the study area to discuss management-related issues and resolve possible conflicts over collection rights. They also worked with communities in other areas, sharing the study results and investigating the possibility of similar co-management efforts there. They shared study information with the authorities as well, since any official recognition of these community-based management practices will require government familiarity and involvement. At the close of the AquaFish study, CIDEA will continue working on bivalve sanitation and depuration under a new two-year grant from European Union.

Unexpected Difficulties

Problems arose over the energy system used in the cockle purification process. Earlier AquaFish mollusk purification research had shown that traditional depuration systems are not feasible. CIDEA thus cooperated with the Central American Energy Alliance in a project involving the use of photovoltaic solar power to supply the energy and ultraviolet radiation to treat the black-cockle bivalves. This updated system of solar panels and water sterilization equipment worked effectively in the purification process.

Researcher communication with local shell fishers was complicated by the fact that a substantial number of women fishers in the study area cannot read or write. During the training process, the problem was addressed by using methods such as skits to illustrate cockle sampling techniques.

Collaboration

An important goal of this study was to help the approximately 2,600 people in the Aserradores Estuary who suffered economic hardship during the four months in which cockle collection is seasonally banned. The AquaFish scientists sought to demonstrate that cockle resources can be more effectively protected, and without prejudice to those relying on these resource for a living – especially women, children, and poor fishers. To this end, AquaFish collaborated with a number of local institutions, including (a) contacts with government fisheries-development such as the Nicaraguan Environment Department (MARENA); (b) meetings with local authorities such as municipal governments in the Aserradores Estuary; and (c) cooperation agreements between the Nicaraguan Fishery and Aquaculture Department and NGOs sponsoring outreach events.

The AquaFish investigation also was supported by the European Union's initiative on the product value-add processes. The alliance for energy and environment partnership played an indispensible role in the implementation of cockle sampling and sanitation. For example, the Central American Energy Alliance helped install a depuration plant and Nicaraguan Agricultural and Forestry Department (MAGFOR) provided the shellfish certifications.

Significant Outcomes

The study showed that fishers with low education and income are able to make sustainable use of resources for their livelihood, based on verbal communal agreements that are unsigned and unmonitored from outside the community. As a byproduct of the AquaFish work, researchers have developed deeper connections with shell-fish institutions throughout Nicaragua, such as in collaboration in the National Strategic Plan for shell-fish development.

Future Study

The researchers say more work now needs to be conducted on pesticide residues in Anadara mollusk tissues and on identifying pollution sources in estuarine areas. Better knowledge is needed of basic cockle biology such as its growth, sexual maturity, and spawning. Beyond that, fishers need more training in credit access, in marketing and project management, and in environmental awareness programs.

Feeding Strategies to Reduce Tilapia Production Costs in Philippines

Case Study of AquaFish Investigation 09SFT04NC

Jangho Choi, Lin Qin, and Steven Buccola

Principal Investigator:

Remedios Bolivar, Professor, Freshwater Aquaculture Center (FAC)/ College of Fisheries, Central Luzon State University (CLSU), Science City of Munoz, Nueva Ecija, Philippines.

Introduction

Aquaculture in the Philippines is a high food-security priority, particularly in light of the country's rapidly growing population and continued dependence on fish protein. Feeds clearly are one of the most costly aspects of fish farming, representing as much as 60% to 80% of production cost of small-scale tilapia aquaculture. Feed waste and the escalating cost of fishmeal in commercial diets are the main problem on small fish farms, where feed sources are rapidly declining and feed demand remains high.

AquaFish researchers and scientists from Central Luzon State University and North Carolina State University collaborated in the present investigation to examine whether Nile tilapia feed costs can be contained by: (a) reducing the amount of feed below that used in satiation feeding; (b) reducing feed costs by reducing crude-protein, supplementing amino acids, and replacing expensive fish meal with lower-cost proteins; and (3) reducing feed manufacturing cost by pellet- rather than the more expensive extrusion-processing.

Links to Earlier Research

The team's investigations 07SFT02NC and 07SFT03NC during the AquaFish 2007 - 2009 phase, in which they analyzed feed strategies for reducing tilapia and milkfish production costs, were closely related to the present one. In those studies, limiting nutrient loads from feed waste was found helpful for mitigating fish farming's negative environmental effects.

Experimental Design

In the present 2009-2011 phase, four studies were implemented to examine the best way of reducing feed costs. In each, growth and feed-conversion rates in sex-reversed tilapia fingerlings were monitored and the economic feasibility of the experimental treatments evaluated. Per-pond feed use was recorded daily and water quality weekly. Differences in growth performance, survival rate, and feed consumption were assessed through analysis of variance (ANOVA), and feed costs per kilogram of body examined for profitability.

Study 1 evaluated three feed reduction strategies. Treatment A -- 67% daily feeding until harvest; Treatment B -- 67% daily feeding for 60 days, followed by 50% daily feeding until harvest; and Treatment C -- 67% daily feeding for 60 days, followed by 100% alternate-day feeding until harvest. Each was replicated three times. After 120 days of culture, stocks were harvested and measured for bulk weight, survival rate, and extrapolated gross fish yield [total fish weight at harvest (kg) / pond area (m^2)].

Study 2 tested the grow-out performances of alternative feed ingredients. A – High CP (31%) extruded feed with 6% dietary inclusion of fishmeal – a standard diet in the industry; B -- Low CP (26%) extruded

feed with 6% dietary inclusion of fishmeal, supplemented with amino acids; C – High CP (31%) extruded feed without dietary inclusion of fishmeal; and D – Low CP (26%) extruded feed without dietary inclusion, supplemented with amino acids. Fifty fish samples were collected every two weeks. Individual weights and lengths of 100 fish was obtained at initial and final samplings.

Study 3 evaluated alternative types of pellet binder and feed additives for durability and water stability. Finally, *Study 4* examined sinking-pellet versus floating extruded feeds in tilapia grow-out in ponds. Four fishmeal-free diets were tested: A -- 31% CP floating extruded feed; B -- 31% CP slow-sinking pelleted feed + binders; C -- 26% CP floating extruded feed supplemented with amino acids; and D -- 26% CP slow-sinking pelleted feed supplemented amino acids plus binders. Fish samples were drawn every two weeks by cast net to obtain average weight of fish stocks. Individual weights and lengths of 100 fish were obtained at initial stocking and final sampling.

Unexpected Difficulties

Two unexpected difficulties were encountered in this work. First, delivery of Study #1's formulated feeds from Santeh Feeds Corporation was delayed two weeks. The study's original intention of examining high- and low-crude protein diets with and without pond fertilization thus had to be changed to examining combined feed-reduction strategies in fertilized ponds. The treatments had to be changed as well because of the unavailability of the planned feed formulations. In Study #4, delay of the formulated-feed delivery also required using the same feed regimen in every treatment for two weeks, extending the study's completion date from September 8 to September 23, 2011.

Outcomes

In Study 1, feed-reduction strategies examined differed little in improving tilapia yields (Figure 1).

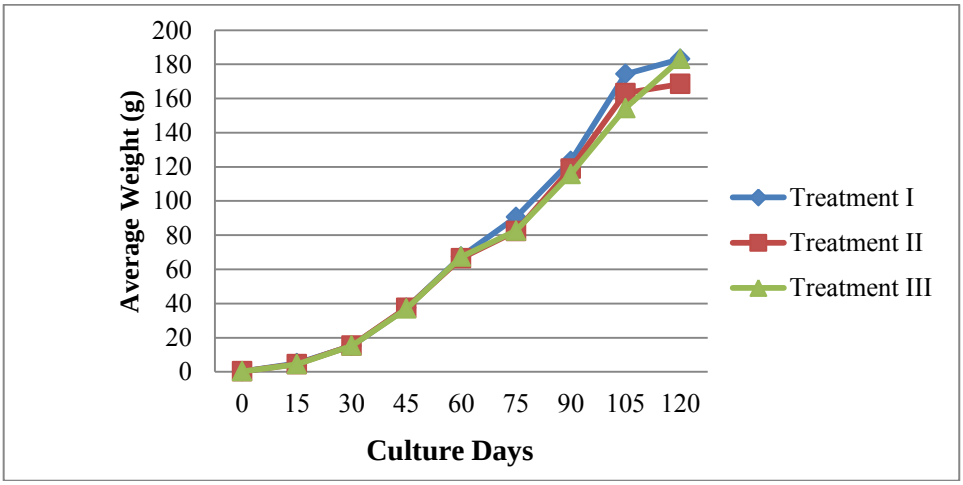


Figure 1. Average weight of Nile tilapia after 120 days of culture in ponds under combined feed-reduction strategies.

Study 2 showed that reducing fishmeal crude protein from the standard 31% CP to 26% CP can generate significant feed-cost savings with no appreciable consequence for Tilapia growth or yield (Figures 2, 3).

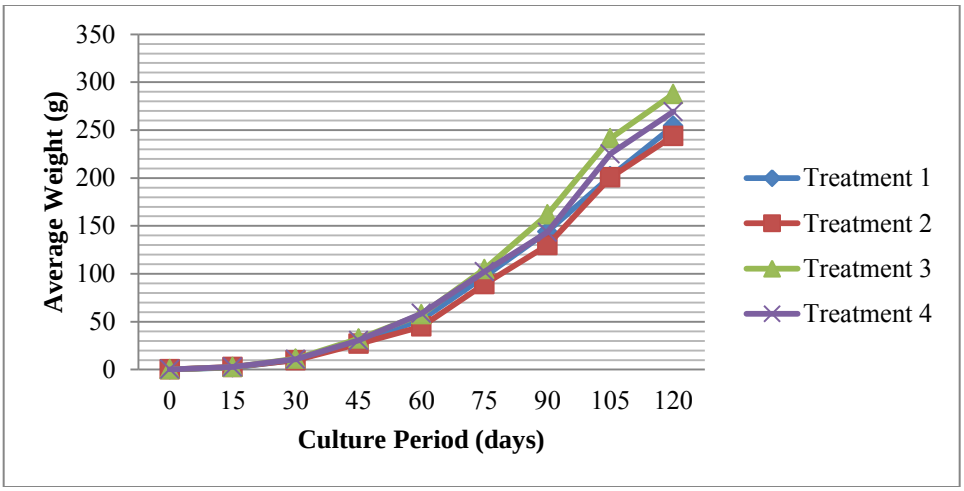


Figure 2. Average weight of Nile tilapia grown in ponds and fed extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal).

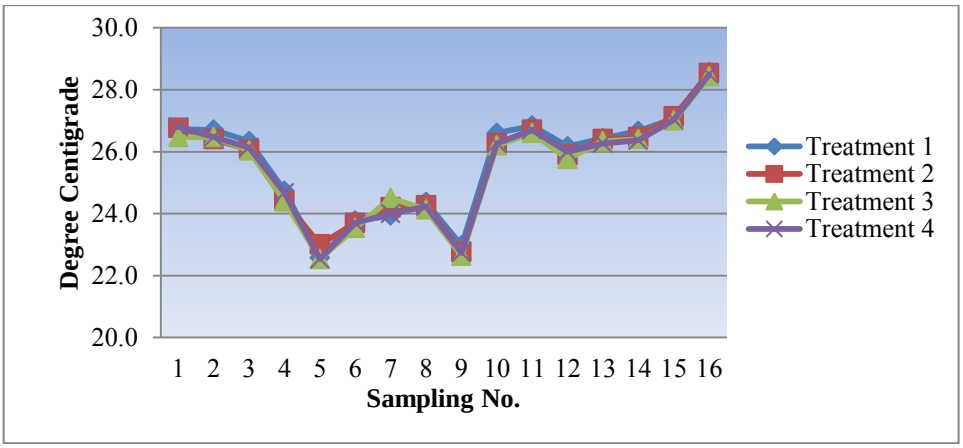


Figure 3. Weekly pond temperatures of Nile tilapia fed extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal).

Study 4 showed that a combination of urea-formaldehyde and gelatin is the most effective binder for maximizing the water stability of pelleted tilapia feed. It also showed that a pelleted fishmeal-free diet in slow-sinking form is more effective than in extruded form, cutting feed costs by 3% with negligible yield impact.

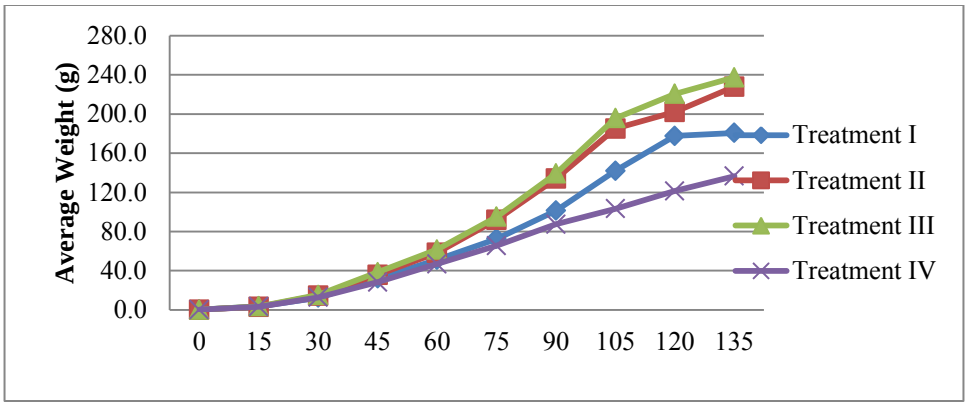


Figure 4. Average weight of Nile tilapia grown in ponds and fed floating extruded or slow-sinking pellet, fishmeal-free grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids.

Communication

This investigation was closely followed by local farmers, who had collaborated earlier with AquaFish and whose feed costs and profitability stood to be improved from the research. Collaborators provided the ponds while the research team provided the fish stock, feeds, and fertilizer, so no additional inputs were necessary. Local feed manufacturing and formulation were coordinated with Santeh Feeds Corporation in Luzon. Government officials, technical personnel at private firms, and academics were invited to the investigation's workshops. Several firms were asked to assist with workshop funding.

Watershed, Water Quality, and Aquaculture in Small South-African Catchments

Case Study of AquaFish Investigation 09WIZ01AU

Jangho Choi, Lin Qin, and Steven Buccola

Principal Investigator:

Khalid Salie, Lecturer, University of Stellenbosch**,** Maitland, South Africa

Introduction

Small impoundments have been built in the region around Stellenbosch, South Africa to deal with the shortage of water supply to agriculture and aquaculture and to a community with growing human population. These small reservoirs provide local fish farmers with enough water to culture trout in cages. However, cage culture operations in these small reservoirs have been problematic on account of rising phytoplankton in aquacultural ponds, which can clog irrigation systems.

The purpose of the present investigation was to evaluate such farm impoundments in the Stellenbosch region. Objectives included examining water use and water quality in ponds with and without aquaculture; a pond's impacts on the local ecosystem, emphasizing biodiversity and wildlife habitat; and the economic and environmental opportunities that are foregone when water is impounded for its present purposes. AquaFish researchers from Auburn University, Stellenbosch University, and Makerere University cooperated in the study.

Earlier Research

Prior to the investigation, the research team had examined alternative ways of improving pond alkalinity and hardness, and has assessed tilapia feeding rates with a combination of artificial and natural feeds. They also had conducted two workshops on water quality and feed management. These projects succeeded in characterizing the variety of aquacultural conditions and practices in the region, which local farmers found useful as management guidelines. They also had served as reference material for governmental policy prescriptions and aquacultural research.

Experimental Design

The present investigation provided measurements of three crucial water-system factors: the quality of the water, the amount and type of downstream pond discharge, and pond effects on environmental biodiversity. Six ponds on five farms were selected: three control ponds without aquaculture and three with aquaculture. Watershed information was obtained through direct observation, mapping, satellite imagery, and discussions with land owners. Average pond depths were determined by sounding.

In order to measure the influence of cage culture operations on pond water quality, water samples were collected by dipping at monthly intervals from January to August 2011. Samples were analyzed for pH, temperature, dissolved oxygen, electrical conductivity, total alkalinity, total hardness, total phosphorus, total ammonia nitrogen, nitrate-nitrogen, nitrite nitrogen, iron, manganese, zinc, and copper.

Pond water balances were constructed analytically to estimate pond runoff from overflow or seepage. Inflow sources in the simulations included direct rainfall, groundwater seepage, and wet-period inflow from ephemeral streams. Outflow consisted of pond overflow, evapo-transpiration, annual discharge, and seepage. Historical data on air temperature, rainfall, and evaporation in the region also had to be considered. Pond water levels were recorded weekly from January 19 to September 1, 2011. Biodiversity was accounted for by noting the species of wetland plants, birds, small mammals, fish in the pond neighborhoods.

Unexpected Difficulties

The AquaFish researchers encountered several unexpected difficulties. Due to the investigation's late start, some farmers who had indicated their availability to serve as study collaborators had cancel their offers. New study sites and collaborators had to be recruited. Other farmer-collaborators did not stay with the planned timeline, and study progress often was inhibited by poor communication and reply delays from them. Several experiments were found to require specialized equipment for which there was no budget. Worst, other equipment was vandalized and stolen, replacement costs of which were especially high in the less-accessible areas. On account of these difficulties, accurate information often was unavailable about a pond's water withdrawals and diversions for irrigation and downstream flow, complicating the development of a detailed water-use budget in those ponds.

Communication

The importance of water resource management and facilitation of multiple water uses is so great that the present research had strong direct implications for farmers and public officials alike. On the other hand, because it ranged over a wide geographic area and involved a large number of interested parties, its logistical difficulties were substantial. Support from fish-farm owners and managers, laborers, the Cape Town Municipality, the Department of Water Affairs, and the Water Research Commission provided crucial support and funding.

Significant Outcome

The researchers found, most importantly, that formation of permanent water surfaces and small wetland areas in fishpond vicinities has enhanced ecosystem complexity and biodiversity. On the other hand, although differences among and between control- and aquaculture-type ponds were not statistically different in this respect, ammonia and nitrate concentrations tend to be greater in aquacultural than in other ponds. The implication is that aquaculture's feed inputs may tend to damage water quality. However, by drastically reducing water levels, crop agricultural irrigation appears to infringe most on the functionality of multipurpose ponds in which fish farmers are involved.

Future Work

This study points clearly to several future research directions. One is to examine the ecological and health status of the region's sediments, including a comparison of long-term sediment conditions in fish- and non-fish farming reservoirs, and an analysis of management practices for minimizing feed waste and improving fish-farm productivity. Attention also needs to be paid to smaller reservoirs' resilience to water quality degradation and to their possibilities for recovering from it during non-farming periods.

Alternative Feeds For Freshwater Aquaculture Species In Vietnam

Sustainable Feed Technology/Study/09SFT01UC

David A. Bengtson
University of Rhode Island
Kingston, Rhode Island, USA

Tran Thi Thanh Hien
Can Tho University
Can Tho, Vietnam

INTRODUCTION

Aquaculture is growing rapidly in Vietnam and has the potential to do the same in Cambodia. Production of pangasiid catfish in the Mekong Delta of Vietnam alone exceeded 1 million metric tons in 2008. While some of the food provided to these fish, especially at the larger commercial farms, is pelleted feed from commercial feed mills, many small farmers still use "trash fish" from the Mekong in preparing feed by hand at the farm. In Cambodia, catfish culture is still at the small-farm stage and trash fish comprise the basic feed for the industry (which is considerably smaller in Cambodia than in Vietnam).

As aquaculture expands in Vietnam and Cambodia, the fish called snakehead is becoming popular to culture because of its high value in the market. There are actually two species currently being cultured, *Channa striata*, the snakehead murrel, and *Channa micropeltes*, the giant snakehead. While culture of these is permitted (and growing) in Vietnam, it is prohibited in Cambodia (except for some experimental work) due to its dependence on small fish in the diet. Catfish culture has available commercial pellet diets, so getting farmers to switch from small fish to pellets is a socioeconomic issue. On the other hand, pelleted diets do not yet exist for snakehead in Vietnam. There have been very few studies conducted on feed and feeding in Cambodia (Heng et al. 2004). There is no tradition of on-farm feed formulation that can be widely used in aquaculture systems. Pond fertilizer techniques are well understood by farmers but organic manures are scarce since they are needed for agricultural crops. The market price for farmed fish, especially in relation to the cost of feed, is a major problem. Prices are very low when fish are plentiful from capture fisheries and consumers prefer wild captured fish to cultured fish.

In the Mekong Delta in Vietnam domesticated snakehead (*Channa micropeltes* and *C. striata*) are fed with small-sized/low value fish (of both marine and freshwater origin). In Cambodia wild giant snakeheads (*Channa micropeltes*) are generally cultured in smaller cages of less than 200 m^3. Feed represents more than 70% of the total operational cost and the main type of feed for wild giant snakehead culture in Cambodia is small-sized/low value fish of freshwater origin (So et al., 2005).
During phase 1 of this project (2007-2009), we determined species composition, size and chemical composition of the main freshwater trash/low-value fish species used as feed for finfish aquaculture in the Mekong Delta of Vietnam. We also developed weaning methods so that small, hatchery-reared snakehead can be quickly adapted to pelleted diets. (When snakehead are collected from the wild by fishermen for aquaculture, it is difficult to impossible to get them to feed on pelleted diets in captivity; the transition must be done in the early life stages.). We then determined that *Channa striata* snakehead survive as well on pelleted diets in which up to 50% of the fish meal has been replaced by soybean meal as they do on pelleted diets made purely of fish meal. On the other hand, growth was equivalent only up to the point of 30% replacement of fish meal with soybean meal, if only the appropriate amino acids are added to the soybean diets; however, that level was increased to 40% replacement when we also added phytase to

break down the phytin in soybean meal. The addition of taurine to the diets did nothing to increase growth above that seen at the 30% soy replacement alone. Next, we determined that *Channa micropeltes* snakehead survive as well on pelleted diets in which up to 50% of the fish meal has been replaced by soybean meal as they do on pelleted diets made purely of fish meal. On the other hand, growth was equivalent only up to the point of 40% replacement of fish meal with soybean meal when we also added phytase to break down the phytin in soybean meal. Finally, we demonstrated that a mixed fish meal, soybean meal and cassava meal diet (with phytase added) can undergo replacement with rice bran at replacement levels up to 30% with no reduction in survival or growth. Cassava is a locally produced crop that may be easier and cheaper to obtain than soybean meal.

The objective of the current (phase 2) portion of this study was to provide information on alternative diets for snakehead, especially those diets that incorporate locally available plant materials, in order to build a long-term sustainable industry. Through an economic analysis of costs of the diets (based on costs of fish meal and plant proteins vs. trash fish) and the risks of the unavailability of trash fish in the future, the information provided from this study will allow decisions to be made about the development of feed mills for local production of diets for the snakehead industry.

To meet the objective, a series of formulated feed experiments were conducted at the wet laboratory and hapas at College of Aquaculture and Fisheries (CAF) of Cantho University (CTU). Furthermore, we conducted on-farm trials in An Giang and Dong Thap provinces to test the optimal formulated feed for snakehead culture from the CTU trials under actual farm conditions. Specific tasks that were conducted were:

(i) Evaluation of the chemical composition of marine trashfish in order to determine its value as a feed ingredient.
(ii) Determine weaning methods with formulated feeds for snakehead *C. micropeltes*
(iii) Conduct pilot trials on weaning methods with both*(Channa striata* and *C. micropeltes* larvae
(iv) Conduct farm trials on grow-out of *Channa striata* fed with trash fish vs formulated feed
(v) Conduct farm trials on grow-out of *Channa micropeltes* fed with trash fish vs formulated feed
(vi) Conduct studies on the replacement of trashfish by rice bran and rice bran + cassava meal in feed for *Channa striata*
(vii) Conduct grow-out of *Channa striata* on demonstration farms to show local farmers the value of utilizing formulated feed
(viii) Conduct a survey of snakehead farmers using formulated feed in An Giang and Dong Thap provinces to determine the reasons for using formulated feed

RESULTS

2.1 Study 1: Evaluate the chemical composition and quality of trashfish

Introduction

In order to conduct some of the trials in this project, it was necessary to know the chemical composition and quality of the trash fish that would be used for feeding the experimental fish. Both fresh-water and marine trash fish are available in Vietnam. The composition and quality of trash fish in phase 1 of this project were analyzed.

Methods

Sampling was done at three different distribution sites and farm sites (An Giang province). Marine trash fish samples were collected at distribution sites and farm sites on the same day, and then stored on ice and

sent to the College of Aquaculture and Fisheries, Can Tho University for analysis of chemical composition (protein, lipid, moisture and mineral) and TVB-N (Total Volatile Base Nitrogen) analysis. Fresh-water trash fish samples were also collected at the distribution sites.

Results and Discussion

The results showed that protein content of marine trash fish was higher, about 17.0%, than that of freshwater trash fish (15.5%); by contrast, lipid content of marine trash fish (2.22%) was lower than that of freshwater fish (6.2%).

Table 2.1.1. Chemical compositions of trash fish based on wet matter (%)

Trashfish	Moisture (%)	Crude ash (%)	Crude Protein (%)	Crude Lipid (%)
Marine trash fish	73.6±1.50	6.93±0.24	17.0±0.19	2.22±0.21
Freshwater trash fish	71.1±0.41	5.50±0.31	15.5±0.27	6.20±0.48

TVB-N values of marine trash-fish samples collected from distribution sites and farm sites were 99.2 ± 12.0 and 119.0 ± 17.8 mgN/100g, respectively (Fig. 2.1). Pike and Hardy (1997) presented evaluation standards for the freshness of fish. TVB-N value must be lower than 14 mgN/100gfor fish to be categorized as fresh, from 14 to 30 mgN/100g to be considered moderately fresh and fish with TVB-N values over 50 mgN/100g are categorized as stale. Our analysis showed that all of marine trash fish samples were in stale condition according to classification of Pike and Hardy (1997). The reason why trash fish samples showed high values of TVB-N is the long storage duration in transportation from the fishermen to the distribution sites (normally three days or more). In fishmeal processing, TVB-N values in trash fish ranged from 22-143 mgN/100g (Pike and Hardy, 1997). Moreover, after three days of storage on ice, there was an increase in TVB-N value in all samples collected from distribution sites and farm sites (159±17.7 and 139±17.3 mgN/100g, respectively). Randomly sampling trash fish for TVB-N analysis, Tran Thi Thanh Hien et al. (2006) indicated that marine trash fish used for Tra catfish were of bad quality, ranging from 84-148 mgN/100g and averaging 113.2 ± 25.6 mgN/100g for 11 samples collected.

Fresh-water trash fish exhibited low TVB-N values (15.7 ± 0.51mgN/100g) on the day of catching and sampling. The samples looked fresh at the time of analysis and fish were collected and analyzed on the days when they were caught. There was an increase in TVB-N value after three days of storage (43.5 ± 1.1 mgN/100g). According to classification of Pike and Hardy (1997) the fresh-water trash fish were therefore still fresh after three days of storage.

2.2. Study 2: Pilot trials on weaning method using formulated feeds for snakehead larvae

Introduction

First feeding is one of the critical periods in fish larval rearing. Zooplankton such as *Brachionus, Moina* and *Daphnia* are frequently used as food resources in fresh-water larviculture and for ornamental fish. They contain a broad spectrum of digestive enzymes such as proteinase, peptidase, amylase, lipase and even cellulase that can serve as exo-enzymes in the gut of the fish larvae (Lavens and Sorgeloos 1996). The quantity and quality of food given, including the types of food used in each of the developmental stages, can also be critical in larval rearing and most importantly can affect economic aspects. Larval rearing has been successful for freshwater and marine fish larvae using brine shrimp *Artemia* sp (Léger et al., 1986), for walking catfish *Clarias macroceplalus* using *Moina* (Fermine et al., 1991) or for European

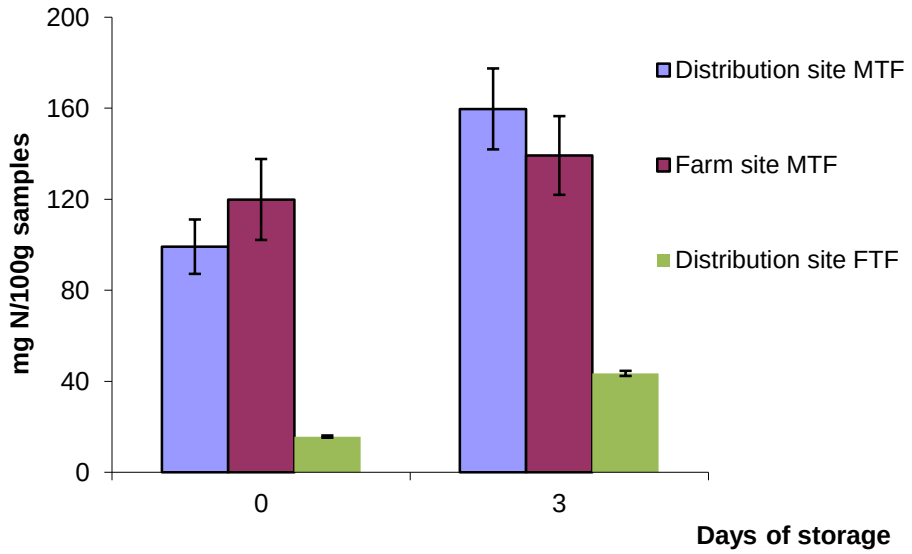


Fig 2.1.1. The freshness of marine trash-fish (MTF) samples collected from distribution and farm sites and fresh water trash fish (FTF) samples collected at distribution sites (n=3). Bars indicate standard errors.

catfish *Silurus glanis* using *Tubifex* worms (Ronyai and Ruttkay, 1990). Some catfish (*Clarias gariepinus* and *Heterobranchus longifilis*) can also be reared exclusively on formulated diet (Appelbaum et al., 1988). However, rearing larvae on formulated diets often resulted in lower growth and survival rate than rearing them on live foods or trash fish. So the present study aims at comparing growth performance and survival rate of *Channa striata* larvae when weaning from live feed to formulated diets.

Methods

After yolk absorption at 3 days after hatching (dah), larvae were fed with *Moina*. The experimental treatments were initiated when the larvae were 10 days after hatch (dah). The experiment was carried out with 9 treatments with three replicates of each treatment. Larvae were stocked at 200 individuals/tank. Trash fish was replaced by experimental feed at 20, 30 or 40 dah at rates of either 10% perday, 10% every two days, or 10% every three days) until larvae were receiving 100% experimental feed per day. Larvae were fed at 7am,10am, 2pm and 5pm.

The temperature varied from 25.92-27.81^{o}C, tending to be higher around noon; dissolved oxygen varied from 4.39 - 5.56 mg/L; pH was around 7; and N-NH_3 and NO_2. were <0.001 and <0.1 ppm. All values are in typical ranges for *C. micropeltes* larval culture in Vietnam.

Table 2.2.1. Environmental parameters

Treat.	Temperature (°C)		Dissolved Oxygen (ppm)		pH		NH_3 (ppm)	NO_2^- (ppm)
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon		
20-1	25.92±0.36	27.79±0.38	5.50±0.32	4.45±0.26	7.47±0.05	7.47±0.06	<0.011	<0.1
20-2	25.98±0.36	27.77±0.37	5.53±0.38	4.39±0.32	7.47±0.04	7.46±0.05	<0.011	<0.1
20-3	26.05±0.38	27.81±0.37	5.53±0.32	4.40±0.42	7.45±0.04	7.46±0.05	<0.011	<0.1
30-1	26.09±0.32	27.79±0.38	5.53±0.34	4.41±0.35	7.45±0.05	7.44±0.07	<0.011	<0.1
30-2	26.06±0.36	27.75±0.38	5.53±0.33	4.47±0.44	7.45±0.04	7.44±0.07	<0.011	<0.1
30-3	25.97±0.33	27.75±0.38	5.47±0.39	4.40±0.46	7.44±0.04	7.45±0.07	<0.011	<0.1
40-1	25.93±0.30	27.76±0.40	5.56±0.36	4.45±0.42	7.45±0.04	7.45±0.05	<0.011	<0.1
40-2	26.03±0.31	27.76±0.38	5.54±0.28	4.46±0.42	7.47±0.04	7.45±0.06	<0.011	<0.1
40-3	25.93±0.28	27.77±0.39	5.54±0.31	4.44±0.52	7.45±0.04	7.43±0.05	<0.011	<0.1

Data measurement and calculation

During the experiment, any mortalities were removed daily and counted. At the end of experiment, all fish were counted and final body weight (FBW, mg) and wet weight gain (WWG, mg) were determined. From those data, we calculated survival rate (SR), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and economic conversion ratio (ECR). In addition, we calculated the mortality rate (based on number of actual mortalities removed from the experiment) and cannibalism rate (based on the initial number of fish stocked minus the number of mortalities removed minus the survivors at the end; i.e., all fish unaccounted for and presumed to have been cannibalized). Differences among treatments were determined by one way ANOVA with means separated using Duncan's Multiple Range test at $p = 0.05$ using SPSS 13.0.

Results

Experiment 1: Pilot trials on weaning method using formulated feeds for snakehead (Channa micropeltes) larvae

The survival rate increased as weaning was delayed (Table 2.2.2).. Within the same weaning time (i.e., 20, 30, or 40 dah), the survival rates tended to increase with increased number of days allowed for weaning, although significant differences were only seen at 40 dah. The non-cannibalism mortality rate was not significantly different among treatments, except for fish weaned at 20 dah over two- or three-day periods. *Channa micropeltes* raised in captivity still retain many characteristics of wild fish. They cannibalize each other if they are not graded weekly. The cannalism rate was highest (44.8%) in treatment 20-1 and differred significantly from the other treatments at $p<0.05$ (Table 2.2.2).

Non-acceptance of formulated feed was observed only among fish weaned at 20 dah (Table 2.2.3). Larvae weaned at 30 and 40 days gave the value of 0% in feed non-acceptance rate. Larvae which did not accept formulated diets became thin and skinny and died gradually or were eaten by larger fish.

Table 2.2.2. Survival rate, non-cannibalism mortality rate, and cannibalism rate. Values (mean±SD) in the same column followed by the same letter are not significantly different.

Treatments	Survival rate (%)	Non-cannibalism mortality rate (%)	Cannibalism rate (%)
20-1	30.5 ± 3.28^{a}	24.7 ± 6.53^{a}	44.8 ± 5.13^{c}
20-2	37.5 ± 11.2^{ab}	47.2 ± 13.6^{bc}	15.3 ± 4.25^{b}
20-3	37.2 ± 9.65^{ab}	54.7 ± 11.5^{c}	8.17 ± 6.17^{ab}
30-1	60.8 ± 11.4^{cd}	29.5 ± 8.26^{ab}	9.67 ± 3.55^{ab}
30-2	63.0 ± 8.19^{cd}	21.8 ± 8.50^{a}	15.2 ± 1.53^{b}
30-3	69.3 ± 19.9^{cd}	18.5 ± 14.9^{a}	12.2 ± 5.01^{ab}
40-1	57.0 ± 18.3^{bc}	30.3 ± 14.7^{ab}	12.7 ± 5.69^{ab}
40-2	73.8 ± 14.3^{cd}	21.2 ± 12.7^{a}	5.00 ± 1.80^{a}
40-3	80.8 ± 2.93^{d}	13.3 ± 4.54^{a}	5.83 ± 2.02^{a}

Figure 2.2.1. Larvae with large fish eating small fish

Table 2.2.3. Rates at which fingerlings did not accept formulated feed**.** Values (mean±SD) in the same column with different letters are significantly different (P<0.05).

Treatments	Rate of feed non-acceptance (%)
20-1	15.7±6.01^{b}
20-2	14.7±1.76^{b}
20-3	10.5±7.81^{b}
30-1	0.00^{a}
30-2	0.00^{a}
30-3	0.00^{a}
40-1	0.00^{a}
40-2	0.00^{a}
40-3	0.00^{a}

Figure 2.2.2. Larvae with thin body did not accept experimental feed. Larvae in treatment 40-3 gave the highest values in weight gain (8.6 g) and and daily weight gain (0.17 g/day) and differed significantly from those in other treatments at $p<0.05$ (Table 2.2.4).

Table 2.2.4. The growth of larvae in the *C. micropeltes* weaning experiment. Values (mean ± SD) in the same column followed by the same letter are not significantly different.

Treatment	Wi (g)	Wf (g)	WG (g)	DWG (g/day)
20-1	0.37±0.01	4.82±0.56^{bc}	4.46±0.56^{bc}	0.09±0.01^{bc}
20-2	0.37±0.01	4.20±0.65^{bc}	3.83±0.65^{bc}	0.08±0.01^{bc}
20-3	0.38±0.01	4.70±0.47^{bc}	4.33±0.48^{bc}	0.09±0.01^{bc}
30-1	0.37±0.01	3.01±0.25^{a}	2.64±0.25^{a}	0.05±0.00^{a}
30-2	0.37±0.00	3.73±0.60^{ab}	3.36±0.60^{ab}	0.07±0.01^{ab}
30-3	0.37±0.01	4.42±0.12^{bc}	4.05±0.11^{bc}	0.08±0.00^{bc}
40-1	0.37±0.01	5.24±0.82^{c}	4.86±0.82^{c}	0.10±0.02^{c}
40-2	0.37±0.01	7.23±0.79^{d}	6.86±0.78^{d}	0.14±0.02^{d}
40-3	0.37±0.01	8.97±1.07^{e}	8.60±1.08^{e}	0.17±0.02^{e}

The size disparity among fish in the different treatments can be seen in Fig. 2.2.3, which groups the fish into 5-g size classes for enumeration.

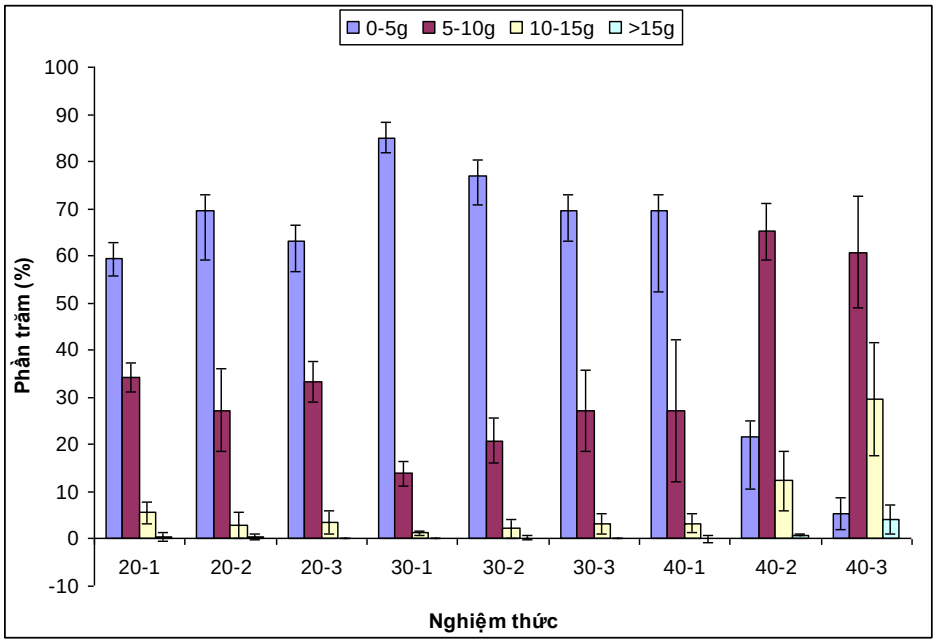


Figure 2.2.3. Size variation of snakehead fed with different diets

Figure 2.2.4. Size disparity of *Channa micropeltes* at the end of experiment

In general, the best weaning strategy that is apparent from this experiment is to wean giant snakehead beginning at 40 dah and using a three-day transition to formulated feed. Since this strategy yielded about 81% survival of the fish, some improvement ot this strategy may be possible, but our results are considered quite acceptable for snakehead culture in Vietnam.

Experiment 2: Pilot trials on weaning method using formulated feeds for snakehead *(Channa striata)* larvae on farms.

Introduction

During phase 1 of this project, we conducted weaning trials with *Channa striata* in the facilities at CTU to determine the optimal strategy for weaning this important species. One critical factor in convincing snakehead farmers to use formulated feed is the availability of snakehead fingerlings that have been weaned to pellets in the hatchery. Wild snakehead or snakehead that have been reared in a hatchery without weaning cannot be weaned to pellets once they are in the grow-out facility. In the current trial, we wanted to test our optimal weaning strategy under actual farm conditions.

Methodology

The experiment was carried out in An Giang and Dong Thap provinces simultaneously at one farm in each province. The fish (6 - 7 g/fish in initial weight) were assigned to 6 hapas (50 m^2/hapa) placed in a 1,000 m^2 pond, 180 fish/m^2 in stocking density. Each treatment was triplicated and experimental period was 10 days.

The experiment contained two treatments. In treatment 1, fish were fed with 100% trash fish during experimental period (control treatment). In treatment 2, on the first day, fish were fed 100% trash fish. Beginning the following day, the percentage of trash fish was reduced at a rate of 10% per day and replaced by formulated feed until trash fish was completely replaced by formulated feed on the 10thday. During the experimental period, fish were fed 3 times per day to satiatation. Fish were weighed and counted on the 10th day to calculate the survival rate (SR) and the growth rate. Temperature, oxygen and pH were measured daily. Water quality parameters were in the normal range for snakehead culture in Vietnam (Table 2.2.5). Temperature in the morning ranged from 28.0 – 29.5 ^oC while temperature in the afternoon was higher (29.5 – 32^oC). There was a small fluctuation in pH between the morning (6.7-7.5) and the afternoon (7.0 – 7.7). Dissolved oxygen was low in the morning (2.5 – 4 ppm) and higher in the afternoon (4.0 – 5.2 ppm).

Table 2.2.5. Temperature (^oC), pH and Oxy (ppm) of Study 1 in An Giang province

Provinces		Temperature (^oC)	pH	Oxy (ppm)
An Giang	Morning	28.5 - 29.5	6.8 - 7.5	3.00 – 4.00
	Afternoon	30.0 - 32.0	7.0 - 7.7	4.50 - 5.20
Dong Thap	Morning	28.0 - 29.5	6.7 - 7.3	2.5 - 4
	Afternoon	29.5 - 32.0	7.1 - 7.7	4.0 - 5.0

Results

Survival and Growth

No significant differences were observed between treatments in either survival rate or daily weight gain ($p>0.05$). Thus, snakehead fingerlings can be weaned by formulated feed in replacing trash fish at the rate of 10%.day^{-1} (Table 2.2.6).

Table 2.2.6. Survival rate (%), body weight (g.fish^{-1}) and daily weight gain (g.day^{-1}) of *Channa striata* fed trash fish (TF) or weaned to formulated feed (FF) at two farms in An Giang and Dong Thap provinces. Data are means of three replicates per treatment ± SE. Means for TF and FF in the same column for a given province with the same superscript in each treatment are not significantly different ($p<0.05$).

Provinces	Diets	SR	Initial weight	Final weight	WG	DWG
An Giang	TF	84.5±1.55^a	7.69±0.20 ^{a}	11.2±0.28^a	3.50± 0.28^a	0.50±0.04^a
	FF	81.7±1.19^a	7.69±0.10 ^{a}	10.9±0.19^a	3.16±0.19^a	0.45±0.03^a
Dong Thap	TF	76.0±2.61^a	6.25±0.04^a	10.6±0.27^a	4.36±0.27^a	0.55±0.03^a
	FF	72.5±0.13^a	6.25±0.08^a	10.4±0.16^a	4.10±0.10^a	0.51±0.01^a

Experiment 3: Weaning formulated feed for giant snakehead *(Channa micropeltes)* fingerling

Introduction

Following the laboratory experiment to determine the optimal strategy for weaning *C. micropeltes* to formulated feed (described above), we wanted to try the strategy on an actual farm. Such trials are necessary to convince farmers that they can wean this species under farm conditions.

Methodology

The experiment was carried out in An Giang province. The fish (1 - 2 g/fish in initial weight) were assigned to 6 hapas (50 m^2/hapa) in a 1,000 m^2 pond, 180 fish/m^2 in stocking density. Each treatment was in triplicate and the experimental period was 30 days.

The experiment contained two treatments. In the treatment 1, fish were fed 100% trash fish during the experimental period (control treatment). In treatment 2, on the first day, fish were fed 100% trash fish. Beginning the following day, the percentage of trash fish was reduced by 10% per day and replaced by formulated feed until trash fish was completely replaced by formulated feed on the 10thday. During the experimental period, fish were fed 3 times per day to sanitation. Fish were weighed and counted after 30 days to calculate the survival rate (SR) and the growth rate. Temperature, oxygen and pH were measureddaily. Environmental factors were in a suitable range for snakehead fingerlings. Temperature in the morning ranged from 28.0 – 29.5 ^{o}C while temperature in the afternoon was higher (29.5 – 32^{o}C) (Table 2.2.7). There was a small fluctuation in pH between the morning (6.8-7.5) and the afternoon (7.4 – 7.9). Dissolved oxygen was low in the morning (2.8 – 4.1 ppm) and higher in the afternoon (4.0 – 5.2 ppm).

Table 2.2.7. Temperature (^{o}C), pH and dissolved oxygen (ppm) of experiment 3 in An Giang province

Diets		Temperature (^{o}C)	pH	Oxy (ppm)
Trash fish	Morning	28.0 - 29.5	6.9 - 7.2	2.90 – 4.00
	Afternoon	30.0 - 32.0	7.4 - 7.8	4.00 - 5.20
Formulated feed	Morning	28.5 - 29.5	6.8 - 7.5	2.8 – 4.10
	Afternoon	29.5 - 32.0	7.4 - 7.9	4.2 - 5.0

Results

Survival and growth

Fingerlings that were fed only trash fish had significant greater daily weight gain compared to fish weaned from trash fish to formulated feed ($p<0.05$). However, there was no significant difference between treatments in the survival rate ($p>0.05$). Giant snakehead fingerlings can be weaned by formulated feed in replacing trash fish in the rate 10%.day^{-1} which reduce the dependence on trash fish supply, although farmers should expect that the growth rate of the fish will be reduced, at least temporarily.

Table 2.2.8. Survival rate (%), body weight (g.fish^{-1}) and daily weight gain (g.day^{-1}) of *Channa micropeltes* fed either trash fish for 30 days or weaned from trash fish to formulated feed over 10 days and then grown an additional 20 days. Data are means of three replicates per treatment ± SE. Means in the same column followed by the same superscript are not significantly different.

Diets	Initial weight	Final weight	WG	DWG	SR
Trash fish	1.51±0.87^{a}	15.3±8.84^{b}	13.8±7.97^{b}	0.34±0.19^{b}	89.1±51.4^{a}
Formulated feed	1.51±0.87^{a}	9.97±5.76^{a}	8.46±4.89^{a}	0.21±0.12^{a}	91.1±52.6^{a}

2.3 Study 3: Farm trials on grow-out of *Channa striata* fed with trash fish vs formulated feed

Introduction

In phase 1 of this project, we developed formulated feeds for *Channa striata* in which a significant amount of the fish meal was replaced by soybean meal and local ingredients like cassava meal and rice bran. This diet development was based on laboratory feeding trials in tanks followed by larger scale feeding trials in hapas in ponds at CTU. In the current phase of the project, we wanted to demonstrate the effectiveness of these diets on actual snakehead farms.

Methodology

The experiment was carried out in An Giang and Dong Thap provinces simultaneously. The test was set up with 6 hapas (50m^2/hapa) with a stocking density of 100 fingerlings/m^2. Hapas were placed in 2 ponds, each500 m^2. In pond 1, snakehead fingerlings (12-13g/fish) in the three hapas (5x10 m in size) were fed marine trash fish (control treatment) for 6 months. In pond 2, snakehead fingerlings (12-13g/fish) in the three 3 hapas (5x10 m in size) were fed formulated feed. In the two first months, snakehead fingerlings were fed by diet named CTU- CRSP 1 (44%CP). In the third month, fish were fed by diet CTU- CRSP 2 (41%CP) and CTU- CRSP 3 (38%CP) was used for the two last months. The formulation of three formulated diets is given in Table 2.3.1.

Formulated feed was made from main ingredients such as Kien Giang fish meal, defatted soybean meal, cassava meal, dried rice-bran. All diets were made in an extruding pellet mill at CTU. Trash fish was marine trash fish bought from markets andwas chopped up before feeding.

Table 2.3.1. The formulation of three formulated feed diets (% of dry matter basis) used in the farm trials with *Channa striata.* Note: 1 USD = 20,600 VND

Ingredients	44 % CP	41% CP	38 % CP
Fish meal	32.76	30.21	27.66
Soybean meal	31.87	29.39	26.91
Dried rice-bran	20.00	20.00	20.00
Cassava meal	7.12	11.99	16.86
Premix Vitamin	1.00	1.00	1.00
Premix mineral	1.00	1.00	1.00
Fish oil	3.32	3.48	3.64
Binder	1.90	1.90	1.90
Lysine	0.40	0.44	0.46
Methionine	0.28	0.28	0.28
Threonine	0.41	0.40	0.39
Phytase	0.02	0.02	0.02
Total	100	100	100
Price (USD)	0.94	0.92	0.90

Sampling

The amount of feed used daily was recorded. . The following water quality parameters were measured monthly: Transparency (Secchi disk), pH, dissolved oxygen, NH_3, NO_2^-. Growth was recorded monthly by weighing 30 fish/hapa. At the end of the experiment, a sensory evaluation was conducted to compare the fillet quality of experimental fish and wild fish.

Data calculations

(1) Chemical composition of the experimental feeds (see below)

(2) Survival rate (%) = (Number of fish end of experiment/number of initial fish) x 100

(3) Weight gain (WG) (g) = Final body weight – Initial body weight

(4) Daily weight gain (DWG) ($g.day^{-1}$) = [(Final body weight – Initial body weight)/ duration of the experiment]

(5) Feed Conversion Ratio (FCR)

FCR (wet) = Feed intake (wet) / Weight gain

FCR (dry) = Feed intake (dry) / Weight gain

(6) PER = (Final body weight – Initial body weight) / Protein intake

(7) Abnormal (humpback) rate (%)= 100%*(Number of abnormal fish/total fish)

(8) Profit (USD)= Total income – total cost

(9) Profit ratio (%) = 100*(profit/total cost)

(10) Sensory test of fillets (see below)

Chemical analysis

Feed was analyzed for chemical composition: moisture, crude protein (CP), crude lipid (CL), crude fiber (CF), nitrogen free extracts (NFE) and gross energy, according to AOAC (2000) methods. Loss on drying was used to determine moisture content; protein (N x 6.25) was determined by Kjeldahl method; lipid was determined by Soxhlet method; crude fiber was determined by acid and base hydrolysis; and gross energy was determined by bomb calorimeter. Carbohydrate-NFE = 100 - (CP + CL + CF). Fish samples collected at the beginning and end of the experiment were also analyzed for moisture, crude protein, crude lipid, crude ash and nitrogen free extracts.

Sensory evaluation

At the end of the experiment, all fish were killed, filleted and washed, then they were steamed for 3 minutes. First, these fish were used to determine the difference in the quality of fish fillet between the control and experimental groups by triangle test (2 controls and 1 sample) with three replacements per test. And the control sample was the snakehead which were bought at the local market. There were two samples named trash-fish and formulated feed.

If less than 6 out of 9 detected the odd sample correctly, we determined that there was no significant difference and therefore no need to conduct a sensory test. A pair test was run if there was any difference in any sensory attributes for texture or taste even if they were minor – called a 'descriptive pair test'. On the other hand, if 7 out of 9 people detected the odd sample correctly, there was a significant difference at $P<0.01$ or 6 out of 9 $P<0.05$. In this case, it was necessary to do a comprehensive pair test on appearance, texture and taste. A pair test is hedonic and scored on an intensity scale (1-9 points) on appearance such as liking (1, least like – 5, o.k. – 9, like very much), whiteness (1, dark – 5,medium – 9, very white), and structural integrity (uniformity: 1, very irregular – 5, medium – 9, very uniform); taste, for example liking (1, least like – 5, o.k. – 9, like very much); snakehead-like taste (1, very little – 5, o.k. – 9, very much)' presence of objectionable taste (yes or no) and presence of objectionable odor (yes or no); texture, for instance, liking (1, least like – 5, o.k. – 9, like very much); firmness (1, very soft – 5, medium – 9, very firm); moistness (1, very dry – 5, medium – 9, very moist); chewiness (1, mushy – 5, medium – 9, very chewy); and flakiness(1, least or rubbery – 5, medium – 9, very flaky). Mean values of results in different treatments were compared by paired sample t-test using SPSS 13.0 software. Treatment effects were considered with the significance level at $P< 0.05$.

Results

Experimental diets

Proximate analyses of the experimental diets are given in Table 2.3.2.

Table 2.3.2. Proximate analysis of experimental diets (% dry matter basis) used in the farm trial with *Channa striata*. MTF refers to the marine trash fish diet used against which the formulated feeds were tested.

Composition (%)	44 % CP	41% CP	38 % CP	MTF*
Dry matter	90.0	90.5	90.3	26.4
Crude protein	44.1	41.1	38.1	59.1
Crude lipid	9.5	9.7	9.6	9.85
Nitrogen free extract	28.3	32.6	35.9	-
Crude ash	11.6	10.2	10.0	27.8
Crude Fibre	6.50	6.40	6.40	-

Proximate compositions of the formulated feeds were similar to the theoretical levels in the diet formulations diet while trash fish had higher protein level (59.1%) in dry basic matter.

Water quality parameters

Water quality parameters are presented in Table 2.3.3. There was some variation in water quality parameters between the two experimental ponds in each province. In particular, dissolved oxygen levels were lower in the ponds fed trash fish than they were in the ponds fed formulated feed. Trash fish is generally considered to pollute the water in which the fish to which it is fed are held and our observations may indicate that more oxygen is being used to break down the organic matter in the trash fish-fed ponds than in the formulated diet-fed ponds.

Table 2.3.3. Water quality parameters in An Giang and Dong Thap provinces

Provinces	Diets	Temperature (^oC)	pH	DO (ppm)	Transparency (cm)	NH_3 (mgL^{-1})	NO_2^- (mgL^{-1})
An Giang	Trash fish	29.0 - 31.8	7.1 - 7.8	2.5 – 3.0	22 – 30	0.01 – 0.08	0.01 – 0.05
	Formulated feed	29.0 - 31.8	7.0 - 7.5	3.0 - 4.5	20 - 25	0.01 – 0.02	0.01 – 0.03
Dong Thap	Trash fish	29 - 31.8	7 - 7.3	2.2 – 3.2	20 - 25	0.02 – 0.06	0.01 – 0.05
	Formulated feed	28.7 - 31.5	7 - 7.5	3.5 – 4.5	18 - 22	0.01 – 0.02	0.01 – 0.02

Survival and growth

After 6 months of culture in An Giang province, the average final weight of fish fed fed with formulated diet (403 ± 2.21g) was significantly higher than that of fish fed with trash fish (391 ± 3.32g) (Fig. 2.3.1), as was daily weight gain (2.75±0.02 $g.day^{-1}$ and 2.67±0.02 $g.day^{-1}$ respectively) (Table 2.3.4). After 4 months of culture in Dong Thap province, the average final weight of fish fed with trash fish (136 $\pm$ 10 g) was significantly lower than that of fish fed with formulated diet (199 $\pm$ 3 g)(Fig, 2.3.2), as was daily weight gain (1.15 $\pm$ 0.10 g/day and 1.74 $\pm$ 0.02 g/day, respectively) (Table 2.3.4).

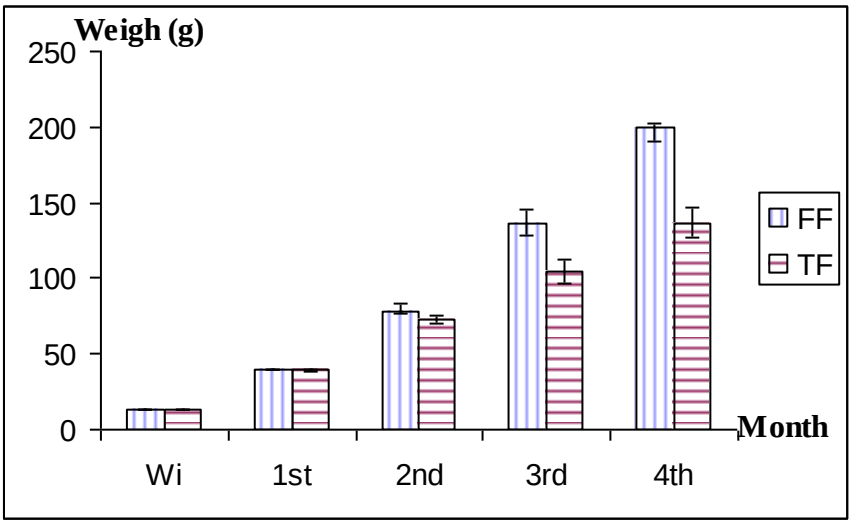


Figure 2.3.1. The growth performance of snakehead throughout the crop in An Giang province

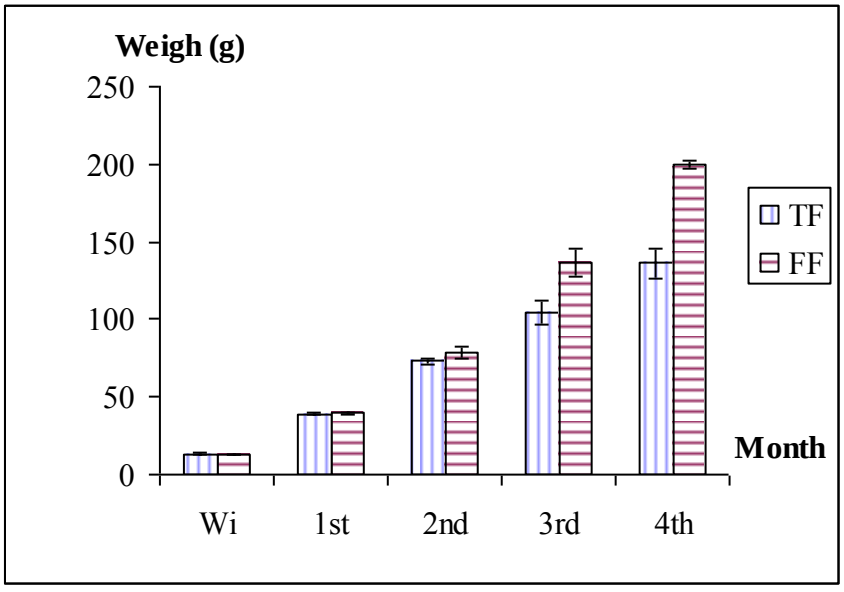


Figure 2.3.2. The growth performance of snakehead throughout the crop in Dong Thap province

The survival rate in the experiment showed no significance between fish fed formulated feed and trash fish in the two provinces ($p<0.05$) (74.8±0.55% and 73.3±0.32%, An Giang province; 79.7±2.67% and 78.5±3.44%, Dong Thap province) (Table 2.3.4).

Table 2.3.4. Survival rate (%), feed conversion ratio (wet and dry basic matter), protein efficiency ratio (protein. weight gain^{-1}) and abnormal rate (%) of *Channa striata* fed experimental diets of experiment 2 in An Giang and Dong Thap province.

Provinces	Diets	SR	FCR (wet)	FCR (dry)	PER	Abnormal rate (%)	DWG
AG	TF	74.8±0.55^{a}	4.45±0.07^{b}	1.12±0.02^{b}	1.78±0.03^{b}	1.26±0.16^{a}	2.67±0.02^{a}
	FF	73.3±0.32^{a}	1.44±0.03^{a}	1.29±0.03^{a}	1.56±0.03^{a}	20.1±1.83^{b}	2.75±0.02^{b}
DT	TF	78.5±3.44^{a}	3.72 ± 0.10^{b}	1.10 ± 0.04^{a}	1.56±0.06^{b}	00	1.15±0.10^{a}
	FF	79.7±2.67^{a}	1.59 ± 0.04^{a}	1.59 ± 0.04^{b}	1.34±0.04^{a}	00	1.74±0.02^{b}

Note: TF: trash fishFF: formulated feed

Abnormal rate from fish fed formulated diet (20.1 ± 1.83%) was significantly higher than that of fish fed the trash fish diet (1.26 ± 0.16%) ($p<0.05$) in the trial at An Giang province; however,. we did not observe any abnormal fish in the trial in Dong Thap province (Table 2.3.4).

FCR on a wet matter basis from treatment fed trash fish showed significantly higher values (4.45 ± 0.07 in An Giang province and 3.72 ± 0.10 in Dong Thap province) than did the treatments fed formulated feed (1.44 ± 0.03 in An Giang province and 1.59 ± 0.04 in Dong Thap province). However, calculating on a dry matter basis, FCR of trash fish diet was significantly lower (1.12 ± 0.02 in An Giang province and 1.10 ± 0.04 in Dong Thap province) than that of formulated feed (1.29 ± 0.03 in An Giang province and 1.59 ± 0.04 in Dong Thap province) in both provinces. (It should be noted, though, that trash fish is purchased on a wet matter basis, not on a dry matter basis.) In addition, PER from the treatment fed trash fish showed significantly higher values than did the treatment fed formulated feed ($p<0.05$) (1.78 ± 0.03 and 1.56 ± 0.03, An Giang province; 1.56 ± 0.06 and 1.34 ± 0.04, Dong Thap province). However, the daily weight gain of the fish fed formulated feed was significantly higher than that of fish fed trash fish (2.75 ± 0.02 and 2.67 ± 0.02, An Giang province; 1.74 ± 0.02 and 1.15 ± 0.10, Dong Thap province).

Table 2.3.5. Production and yield of *Channa striata* fed experimental diets in An Giang and Dong Thap provinces.

Provinces	Diets	Production (kg)	Yield (kg/m^2)
An Giang	TF	1462 ± 8.01^{a}	29.2 ± 0.16^{a}
	FF	1476 ± 12.6^{a}	29.5 ± 0.25^{a}
Dong Thap	TF	533 ± 30.7^{a}	10.7 ± 0.61^{a}
	FF	789 ± 13.7^{b}	15.8 ± 0.27^{b}

There was no significant difference in production or yield between ponds fed formulated feed or trash fish for snakehead culture in An Giang province ($p>0.05$) (Table 2.3.5). In Dong Thap province, production and yield in ponds given formulated feed were significantly higher than those in ponds fed trash fish (Table 2.3.5).

Economics

The feed cost made up the biggest variable cost for snakehead culture in these trials, whereas costs of labor, fingerlings and chemicals were relatively minor (Figs. 23.4 and 2.3.5)

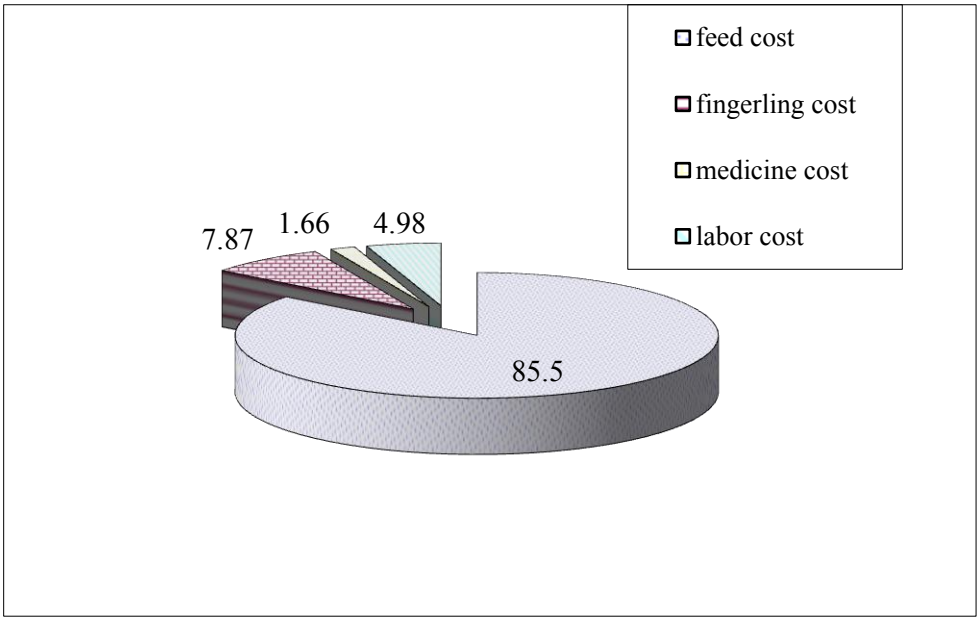


Figure 2.3.3. Breakdown of variable costs as percentages of total cost for the ponds fed formulated feed in the farm trials with *Channa striata*

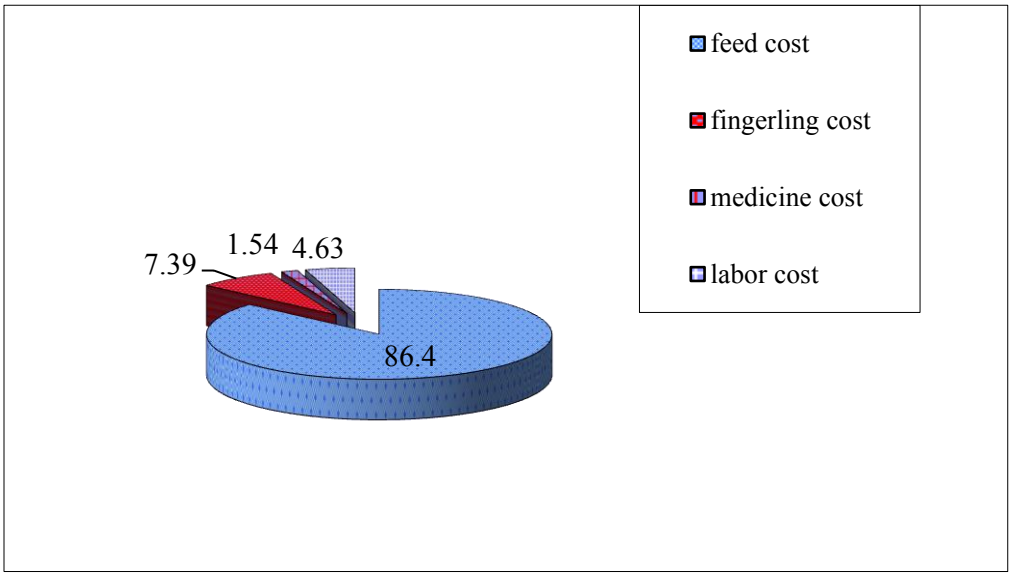


Figure 2.3.4. Breakdown of variable costs as percentages of total cost for the ponds fed trash fish in the farm trials with *Channa striata*

Profit from ponds fed trash fish were much lower than that from ponds fed formulated feed and there was a significant difference between treaments ($p<0.05$) in both provinces (Table 2.3.6). Profit in both treatments in An Giang province were much lower than those in Dong Thap province. This was primarily due to the lower income received for both types of fish in Dong Thap because they were smaller sized.

Table 2.3.6. Economics of experimental snakehead culture in An Giang and Dong Thap province on a per-hapa and per-meter-squared basis. Note: Crop duration was 6 months for An Giang province and 4 months for Dong Thap province.

Provinces	Diets	Total cost (million VND/hapa)	Total income (million VND/hapa)	Profit (million VND/hapa)
An Giang	TF	14.3 ± 0.13^{b}	20.6 ± 0.11^{a}	6.37 ± 0.21^{a}
	FF	13.4 ± 0.14^{a}	20.8 ± 0.18^{a}	7.43 ± 0.32^{b}
Dong Thap	TF	5.97 ± 0.16^{a}	11.5 ± 0.66^{a}	5.54 ± 0.51^{a}
	FF	9.04 ± 0.16^{b}	17.0 ± 0.30^{b}	8.01 ± 0.38^{b}

Provinces	Diets	Total cost (million VND/m^2)	Total income (million VND/m^2)	Profit (million VND/m^2)
An Giang	TF	0.95 ± 0.01^{b}	1.37 ± 0.01^{a}	0.42 ± 0.01^{a}
	FF	0.89 ± 0.01^{a}	1.39 ± 0.01^{a}	0.50 ± 0.02^{b}
Dong Thap	TF	0.25 ± 0.01^{a}	0.48 ± 0.03^{a}	0.23 ± 0.02^{a}
	FF	0.38 ± 0.01^{b}	0.71 ± 0.01^{b}	0.33 ± 0.02^{b}

Table 2.3.7. Economics of experimental snakehead culture in An Giang and Dong Thap province on a per kg fish basis. Note: Crop durationwas 6 months for An Giang province and 4 months for Dong Thap province

Provinces	**Diets**	**Total cost (thousand VND/kg fish)**	**Total income (thousand VND/kg fish)**	**Profit (thousand VND/kg fish)**
An Giang	TF	32.5 ± 0.41^{b}	47.0 ± 0.00	14.5 ± 0.41^{a}
	FF	30.5 ± 0.62^{a}	47.0 ± 0.00	16.5 ± 0.62^{b}
Dong Thap	TF	24.0 ± 0.62^{a}	45.0 ± 0.00	21.0 ± 0.62^{a}
	FF	23.5 ± 0.62^{a}	45.0 ± 0.00	21.5 ± 0.62^{a}

Fish quality

Sensory analysis

In appearance, both fish fed trashfish and formulated feed received scores of approximately 4 to 5, meaning that the fillets were passable or fairly likable for liking; medium or rather white for whiteness; except structural integrity, for which the scores were nearly 7- relatively uniform. In taste, the fish fillet had snakehead-like taste without the presence of objectionable taste and odor. In texture examination, for liking, the score was from 5 to 6, from rather not like – passable – fairly like.For firmness, the scores were 4-5, relatively soft – medium fish fillet. The fillet moistness was judged to be rather dry to medium (not dry and not moist). The fillet chewiness and flakiness was fairly mushy and relatively rubbery or medium (not mushy and not chewy; not rubbery and not flaky).

The result showed that there was no significant difference between paired samples in triangle tests (less than 6 out of 9 people detected the odd sample correctly). These samples were then subjected to "descriptive" pair tests, with the result that the quality of fish fillet samples from the two treatments did not significantly differ.

Table 2.3.8. Triangle test for difference (Number from a 9-person sample who detected the odd sample correctly

TF	FF
2.67 ± 0.33	4.33 ± 0.33

Table 2.3.9. *Channa striata* sensory analysis. Data are mean ± SE.

Content	Scores	
	TF	FF
Appearance		
Liking	4.70 ± 0.07	4.96 ± 0.13
Whiteness	4.59 ± 0.07	4.48 ± 0.04
Structural integrity	7.00 ± 0.00	6.93 ± 0.07
Taste		
Liking	4.30 ± 0.10	4.70 ± 0.10
Snakehead-like taste	4.56 ± 0.11	5.81 ± 0.23
Presence of objectionable taste	No	No
Presence of objectionable odor	No	No
Texture		
Liking	5.30 ± 0.10	5.89 ± 0.19
Firmness	5.41 ± 0.07	4.93 ± 0.10
Moistness	3.74 ± 0.07	5.04 ± 0.36
Chewiness	5.63 ± 0.04	5.67 ± 0.06
Flakiness	3.70 ± 0.07	4.04 ± 0.13

In summary, snakehead fillet quality was fairly like and did not significantly differ between samples in triangle tests. In desciptive pair tests, there was also no significant difference between samples. So, the diets did not affect on the quality of fish fillet for fish in these farm trials.The results confirmed that trash fish can be replaced by formulated feed for snakehead culture.

2.4 Study 4: Farm trials on grow-out of *Channa micropeltes* fed with with trash fish vs Formulated feed

Introduction

Just as for *Channa striata*, it is important to demonstrate the effectiveness in actual farm trials of the formulated diets developed in phase 1 of this project for *Channa micropeltes*.

Methodology

The experiment was carried out in An Giang province. The test was set up with 6 hapas (50m^2/hapa) placed in one pond (700 m^2)with a stocking density of 100 fingerlings/m^2. Giant snakehead fingerlings (12 - 13g/fish) in three hapas (5x10 m in size) were fed marine trash fish (control treatment) for 5 months. Fingerlings (12-13g/fish) in the other three hapas (5x10 m in size) were fed formulated feed. In the two first months, snakehead fingerlings were fed the diet named CTU- CRSP- 1 (44%CP). In the third month, fish were fed by diet CTU- CRSP- 2 (41%CP) and CTU- CRSP- 3 (38%CP) for the two last months. Experimental diets, sampling, data calculation and analysis were done the same way as experiments on snakehead experiments

Results

The experiment will be completed on 15 September

2.5 Study 5: Replacing trash fish by rice bran and rice bran + cassava meal in snakehead *Channa striata* feed

Introduction

Although hatchery-reared snakehead that have been weaned to pellets are available in Vietnam, such fish are not available in Cambodia and it will take some time before Cambodian hatcheries are fully functional and the snakehead have been domesticated there. In the short term in Cambodia, it will be necessary to use moist diets based on trash fish and other ingredients, such as rice bran and cassava meal. Rice bran is available and abundant in Mekong Delta. Using this ingredient will reduce feed cost for fish and animal production. Actually, it has often been used in formulated fish feed such as commercial feed (30-40% of rice bran has been used) and home-made feed (60-70%) for some species. Rice bran contains vitamins A, D, E, B1, and B2 at levels higher than those in corn, and rather high protein 8.34-16.3% (Hien et al., 2009).

Cassava meal is acheap source of carbohydrates and serves as a good binder in fish feed. According to Hien (2009), the dry matter digestibility of cassava is approximately 83.3%, high compared to other carbohydrate sources.

Methodology

Figure 2.5.1. Experimental hapas

Experimental fish

Before starting the experiments, all the fish were reared in 2000-L round tanks and were fed with trash fish combined with pellet diets for 2 weeks. Replacement of trash fish by pellet feed was applied gradually at a rate of 10% day-1 until 100% of trash fish was substituted by pellet feed.

Experimental design

There are two experiments in this study. The first is replacing marine trash-fish (MTF) by rice bran and cassava meal. The second is replacing freshwater trash-fish (FTF) by those ingredients. Both experiments were conducted with 11 diet treatments consisting of a control diet formulated pellet (FP) which concluded fish meal, soybean meal, rice bran and cassava meal in the optimal combination as determined

from experiments in phase 1; marine trash-fish (MTF); freshwater trash-fish (FTF); and others which were mixed with different ratios of MTF or FTF, rice bran (RB) and RB + cassava meal (CM) (Tables 2.5.1 and 2.5.2) Each treatment had three replicates.

The 33 hapas (1x1x2m/hapa) used in each experiment were fixed in a pond in CAF, Cantho University (Fig. 2.5.1). Fifty snakehead fingerlings (3.7 - 3.9 g in initial weight) were assigned randomly in each hapa. Experimental time was 6 weeks.

Table 2.5.1. Composition of 11 experimental diets (%)

Treat	Mixed ratios	FP	MTF	FTF	Replacing ratios of MTF (FTF)	RB	CM
1	Formulated Pellet (FP)	100	-	-		-	-
2	MTF	-	100	-		-	-
3	FTF	-	-	100		-	-
4	MTF (FTF)+RB+CM 80:10:10	-	-		80	10	10
5	MTF (FTF)+RB+CM 70:15:15	-	-		70	15	15
6	MTF (FTF)+RB+CM 60:20:20	-	-		60	20	20
7	MTF (FTF)+RB+CM 50:25:25	-	-		50	25	25
8	MTF (FTF)+RB 80:20	-	-		80	20	-
9	MTF (FTF)+RB 70:30	-			70	30	-
10	MTF (FTF)+RB 60:40	-	-		60	40	-
11	MTF (FTF)+RB 50:50	-	-		50	50	-

Fish were fed until satiation twice daily at 08:00am and 15:00pm. The amount of feed consumed was adjusted on a daily basis and recorded. All hapas were cleaned every two weeks. Total fish weight in each aquarium was determined at the beginning and at the end of experiments. Dead fish were recorded and weighed for calculating feed conversion ratio (FCR).

Water temperature was measured daily and ranged from 27.0–30.1^{o}C. pH and dissolved oxygen were measured weekly and determined to be 6.5–7.6 and 1.9–2.6 ppm in the early morning, and 4.8-6.8 in the afternoon, respectively. No aeration was provided in the ponds.

Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the trial on farm experiments**.**

Results and Discussion

Experiment 1

Marine trash-fish (17.0%) had higher protein content than freshwater trash-fish (15.5%). The lipid content was 2.22% for freshwater and 6.20% for marine trash-fish. Crude protein decreased with increasing replacement of fish meals with rice bran and cassava meal, and diets replacing trash-fish by only rice bran (16.5 – 14.8%) had higher protein content than those replacing TF by both RB and CM (15.5 – 12.5%) (Table 2.1.2).

Table 2.5.2. Chemical compositions of treatment diets basing on wet matter (%)

Treatment diets	Moisture (%)	Crude ash (%)	Crude Protein (%)	Crude Lipid (%)
Formulated Pellet (44%CP)	10.2	10.21	38.80	8.36
MTF	73.6±1.50	6.93±0.24	17.0±0.19	2.22±0.21
FTF	71.1±0.41	5.50±0.31	15.5±0.27	6.20±0.48
MTF+RB+CM 80:10:10	56.8±0.38	6.58±0.21	15.5±0.16	2.97±0.18
MTF+RB+CM 70:15:15	52.8±0.98	6.42±0.09	14.7±0.11	3.22±0.10
MTF+RB+CM 60:20:20	46.0±0.48	6.24±0.07	13.3±0.10	3.41±0.15
MTF+RB+CM 50:25:25	46.1±0.36	6.14±0.07	12.5±0.44	3.79±0.23
MTF+RB 80:20	63.0±0.31	7.07±0.06	16.5±0.11	3.45±0.17
MTF+RB 70:30	54.6±0.03	7.10±0.38	15.8±0.2	4.26±0.08
MTF+RB 60:40	42.8±0.84	7.15±0.03	15.2±0.38	5.37±0.01
MTF+RB 50:50	39.4±0.71	7.19±0.13	14.8±0.17	5.89±0.15

Significantly higher growth rates were obtained with formulated pellet and freshwater trash-fish (0.56 and 0.57 g.day^{-1}, respectively) than with the other treatments (Table 2.5.3). Conversely, significantly lower growth was obtained with diets MTF+RB+CM 50:25:25 (0.21 g.day^{-1}) and MTF+RB 50:50 (0.23 g.day^{-1}). This means that the greater amount of marine trash-fish that is replaced, the lower fish growth will be. In addition, snakehead using FTF grew as fast as fish fed by formulated pellet and faster than fish fed by MTF.

Table 2.5.3. The growth of *Channa striata* fingerlings

Treatments	Wi	Wf	DWG	SGR	SR
FP (44%CP)	3.79±0.01^{a}	27.3±0.25^{e}	0.56±0.01^{e}	4.70±0.02^{c}	91.0±1.73^{d}
MTF	3.75±0.02^{a}	19.0±0.13^{c}	0.36±0.00^{c}	4.66±1.17^{c}	79.0±5.20^{cd}
FTF	3.75±0.01^{a}	27.9±1.09^{e}	0.57±0.03^{e}	4.78±0.10^{c}	68.0±3.06^{abc}
MTF+RB+CM 80:10:10	3.80±0.03^{a}	21.3±0.89^{d}	0.42±0.02^{d}	4.57±0.04^{bc}	70.0±1.15^{abc}
MTF+RB+CM 70:15:15	3.78±0.02^{a}	17.6±1.18b^{c}	0.33±0.03^{bc}	3.84±0.27^{abc}	63.3±6.77^{ab}
MTF+RB+CM 60:20:20	3.76±0.02^{a}	14.8±0.51^{ab}	0.26±0.01^{ab}	3.57±0.31^{abc}	77.0±1.73^{bc}
MTF+RB+CM 50:25:25	3.78±0.01^{a}	12.6±0.43^{a}	0.21±0.01^{a}	2.87±0.09^{a}	66.7±2.40^{abc}
MTF+RB 80:20	3.77±0.02^{a}	14.5±0.25^{ab}	0.26±0.01^{ab}	3.20±0.05^{a}	66.0±3.06^{abc}
MTF+RB 70:30	3.79±0.02^{a}	18.5±1.08^{c}	0.35±0.03^{c}	3.77±0.13^{abc}	62.9±8.86^{ab}
MTF+RB 60:40	3.77±0.00^{a}	16.0±0.26^{b}	0.29±0.01^{b}	3.34±0.15^{ab}	56.0±2.31^{a}
MTF+RB 50:50	3.78±0.02^{a}	13.2±0.93^{a}	0.23±0.02^{a}	2.70±0.32^{a}	58.0±5.29^{a}

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different (P<0.05).

Initial mean weight – Wi (g), final mean weight – Wf (g), daily weight gain – DWG (g.day^{-1}), special gain rate – SGR (%.day^{-1}) and survival rate – SR (%) of snakehead fed experimental diets (Mean±S.E., n=3) in the experiment 1

Survival rate of *C. striata* was the highest with formulated pellet (91.0%) while the lowest values were with diets replacing MTF by RB 60:40 and 50:50 (56.0 and 58.0%, respectively). It may be that snakehead fed by formulated pellet had reduced risk of contact with pathogens in the trash fish. Compared to results of experiments in phase 1 on *C. striata*, the survival rate in this experiment was high in the formulated diet treatment and fairly high in the treatments MTF (79.0%), FTF (68.0%), and treatments in which MTF was replaced by ingredients (56.0-77.0%).

The lowest FCR was in the FP treatment (1.02) and the highest was in the treatment MTF (6.11) (Table 2.5.4). Otherwise, fish effectively used protein in pellet (2.52) better than that in MTF (0.97). Moreover, feed cost per kg weight gain of fish was lowest in the pellet treatment (18,900 VND/kg fish). This value decreased with increasing replacement ratios of MTF by RB or RB and CM. Furthermore, feed cost for FTF is higher than that for MTF (35,900 and 33,600 VND/kg fish, respectively) because of the high price of FTF.

Table 2.5.4. Feed conversion ratio, protein efficiency ratio, and feed costs for producing one kg weight gain of snakehead fed experimental diets (% of wet matter basis) in the experiment 1

Treatments	FCR	PER	Feed cost/kg weight gain of fish (,000 VND/kg fish)
FP (44%CP)	1.02±0.00^{a}	2.52±0.01^{d}	18.9
MTF	6.11±0.35^{d}	0.97±0.06^{a}	33.6
FTF	4.49±0.34^{b}	1.45±0.11^{bc}	35.9
MTF+RB+CM 80:10:10	4.89±0.26^{bc}	1.33±0.07^{b}	24.5
MTF+RB+CM 70:15:15	4.60±0.30^{b}	1.49±0.10^{bc}	21.8
MTF+RB+CM 60:20:20	4.53±0.47^{b}	1.70±0.17^{c}	20.4
MTF+RB+CM 50:25:25	5.77±0.07^{cd}	1.39±0.02^{bc}	24.5
MTF+RB 80:20	4.63±0.22^{b}	1.32±0.06^{b}	23.6
MTF+RB 70:30	4.33±0.41^{b}	1.49±0.15^{bc}	21.2
MTF+RB 60:40	4.40±0.39^{b}	1.52±0.12^{bc}	20.7
MTF+RB 50:50	4.53±0.28^{b}	1.50±0.09^{bc}	20.4

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different (P<0.05).

Experiment 2

Table 2.5.5. Chemical composition of treatment diets based on wet matter (and dry matter in brackets)

Treatment diets	Moisture (%)	Crude ash (%)	Crude Protein (%)	Crude Lipid (%)
FP (44%CP)	(90.0)	(11.6)	(44.1)	(9.50)
MTF	73.1	70.3 (26.1)	16.2 (60.2)	2.5 (9.29)
FTF	70.5	5.78 (19.6)	15.1 (51.3)	6.9 (23.6)
FTF+RB+CM 80:10:10	60.1	4.36 (10.9)	14.9 (37.2)	7.77 (19.5)
FTF+RB+CM 70:15:15	55.1	5.25 (11.7)	14.3 (31.9)	7.83 (17.4)
FTF+RB+CM 60:20:20	51.1	5.45 (11.1)	13.9 (28.5)	8.06 (16.5)
FTF+RB+CM 50:25:25	42.2	5.46 (9.45)	13.0 (22.4)	8.35 (14.5)
FTF+RB 80:20	58.7	6.12 (14.8)	14.5 (35.1)	8.22 (19.0)
FTF+RB 70:30	50.9	6.62 (13.5)	13.3 (27.0)	8.51 (17.3)
FTF+RB 60:40	43.1	7.43 (13.1)	13.2 (23.1)	9.25 (16.3)
FTF+RB 50:50	39.7	7.72 (12.8)	12.9 (21.4)	9.50 (15.8)

The fish fed diets in which FTF was replaced by both rice bran and cassava meal grew faster than those of diets replaced by only rice bran (Table 2.5.6). The highest growth was in the treatment FTF+RB+CM 80:10:10 (1.58 $g.day^{-1}$) and there was no significant difference ($P>0.05$) between treatments FTF, FTF+RB+CM 80:10:10, and FTF+RB+CM 75:15:15 (1.50, 1.58, and 1.53 $g.day^{-1}$, respectively).

Table 2.5.6. The growth and survival rate of snakehead fed with different diets

Treatments	Wi	Wf	DWG	SGR	SR
FP	3.90±0.001	$60.0±0.56^{e}$	$1.34±0.01^{e}$	$6.51±0.02^{f}$	91.0±1.73
MTF	3.91±0.009	$63.7±1.35^{ef}$	$1.42±0.03^{ef}$	$6.65±0.04^{fg}$	86.0±3.46
FTF	3.91±0.006	$67.0±0.66^{fg}$	$1.50±0.02^{fg}$	$6.76±0.02^{g}$	92.0±2.00
FTF+RB+CM 80:10:10	3.91±0.001	$70.3±3.55^{g}$	$1.58±0.08^{g}$	$6.88±0.12^{g}$	92.0±8.00
FTF+RB+CM 70:15:15	3.90±0.002	$68.0±1.18^{fg}$	$1.53±0.03^{fg}$	$6.80±0.04^{g}$	89.3±5.21
FTF+RB+CM 60:20:20	3.91±0.007	$46.6±2.64^{c}$	$1.02±0.06^{c}$	$5.89±0.13^{d}$	82.7±10.4
FTF+RB+CM 50:25:25	3.90±0.004	$38.2±1.73^{b}$	$0.82±0.04^{b}$	$5.42±0.11^{c}$	90.0±5.77
FTF+RB 80:20	3.90±0.004	$54.3±0.41^{d}$	$1.20±0.01^{d}$	$6.27±0.02^{e}$	91.0±1.73
FTF+RB 70:30	3.90±0.003	$34.1±1.39^{b}$	$0.72±0.03^{b}$	$5.16±0.10^{b}$	97.0±0.58
FTF+RB 60:40	3.91±0.008	$20.4±0.22^{a}$	$0.39±0.01^{a}$	$3.94±0.02^{a}$	93.0±2.89
FTF+RB 50:50	3.91±0.007	$19.0±0.04^{a}$	$0.36±0.00^{a}$	$3.77±0.00^{a}$	90.0±3.46

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different ($P<0.05$).

Initial mean weight - Wi (g), final mean weight - Wf (g), daily weight gain – DWG ($g.day^{-1}$), special gain rate - SGR ($\%.day^{-1}$) and survival rate - SR (%) of snakehead fed experimental diets (Mean±S.E., n=3) in the experiment 2

The lowest FCR was in the FP treatment (1.04) and the highest was in the MTF treatment (7.31). There were no significant differences ($P>0.05$) between treatments FTF, FTF+RB+CM 80:10:10, FTF+RB+CM 75:15:15, FTF+RB 80:20, and FTF+RB 70:30 (4.33, 4.40, 4.62, 4.23 and 4.21, respectively). Protein in formulated pellet was the most effectively used (PER = 2.22) while PER was only 0.85 in MTF. In addition, the lowest feed cost per kg weight gain of fish was in the FP treatment (19,700 VND/kg fish),

the highest was in the MTF treatment (47,500 VND/kg fish) and the second lowest feed cost was in the treatment FTF+RB 70:30 (28,000 VND/kg fish). Compared to results of the experiment 1, feed cost per kg weight gain of fish fede diets containing FTF (28,000 – 35,300 VND/kg fish) was higher than those containing MTF (20,400 – 24,500 VND/kg fish) because FTF is had problems of seasonal availability, so its price was high and fluctuated.

Table 2.5.7. Feed conversion ratio, PER and feed costs for producing one kg weight gain of snakehead fed experimental diets (% of wet matter basis) in the experiment 2.

Treatments	FCR	PER	Feed cost/kg weight gain of fish (,000 VND/kg fish)
FP	1.04±0.00^{a}	2.22±0.01^{d}	19.7
MTF	7.31±0.20^{e}	0.85±0.02^{a}	47.5
FTF	4.33±0.38^{b}	1.55±0.15^{bc}	34.7
FTF+RB+CM 80:10:10	4.40±0.30^{b}	1.54±0.11^{bc}	31.1
FTF+RB+CM 70:15:15	4.62±0.19^{bc}	1.52±0.06^{bc}	30.5
FTF+RB+CM 60:20:20	5.38±0.52^{cd}	1.36±0.12^{bc}	33.0
FTF+RB+CM 50:25:25	5.51±0.10^{cd}	1.40±0.03^{bc}	31.2
FTF+RB 80:20	4.23±0.13^{b}	1.64±0.05^{bc}	30.0
FTF+RB 70:30	4.21±0.54^{b}	1.84±0.21^{cd}	28.0
FTF+RB 60:40	5.79±0.41^{d}	1.32±0.09^{b}	35.9
FTF+RB 50:50	6.14±0.07^{d}	1.64±0.38^{bc}	35.3

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different (P<0.05).

To sum up, the snakehead *Channa striata* more effectively used trash fish from freshwater compared to trash fish from marine sources. The results from quality analysisshowed that fresh-water trash fish exhibited low TVB-N values on the day after they were caught. After three days of storage, the fresh-water trash fish was still fresh, whereas marine trash fish were in stale condition. The formulated pellet which was studied from the phase 1 experiments was also effectively used by this kind of fish. Formulated pellet contributed to reduce the feed cost in snakehead culturing. Moreover, farmers could utilize available local rice bran and freshwater trash-fish through the diet which is 70% freshwater trash-fish and 30% rice bran. In addition, farmers could also use diets MTF+RB+CM 60:20:20 or MTF+RB 50:50 for snakehead culturing.

2.6. Study 6. Grow-out of *Channa striata* on demonstration farms

Introduction

In addition to the experiments that were carried out on farms comparing formulated feed to trash fish, two additional demonstrations of use of formulated feed for snakehead culture were carried out in Dong Thap province.

Methodology

Fingerlings were fed with floating commercial pellets. Pellets fed to snakehead in the 1st, 2nd, and 3rd months contained 44% crude protein and the remain months were 40% CP. Pellets were given to satiation. During the first three months, water was changed at 50% volume every three days and daily in the last months. Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the trial on farm experiments**.**

Results

Observation and sampling for two practical farms

Data for the two ponds used in this demonstration are given in Table 2.6.1 and Figure 2.6.1

Table 2.5.1. Data of two ponds used for demonstration in Dong Thap province

Parameters	Pond
Area (m^2)	1300±71
Density (fish.m^2)	100±0.0
Initial weight (g.fish)	2.44±0.05
Final weight (g.fish)	562±135
Abnormal rate (%)	5.82±3.99
WG (g)	560±135
DWG (g/day)	3.05±0.84
SR (%)	54.6±1.59
FCR	1.30±0.02
Production (ton)	30.6±6.5
Yield (kg/m^2)	22.6±3.63

The result was displayed by mean ± STD

Average of two investigated ponds was 1300 ± 71m2, socking density was 100 fish.m^2. After 6 months of stocking, fish weight was 560 ± 135 (g.fish), survival rate was 54.6 ± 1.59 %, abnormal rate was 5.82 ± 3.99 %. FCR was low, 1.30 ± 0.02. Average production was 30.6 ± 6.5 ton and average yield was 22.6 ± 3.63 kg/m^2.

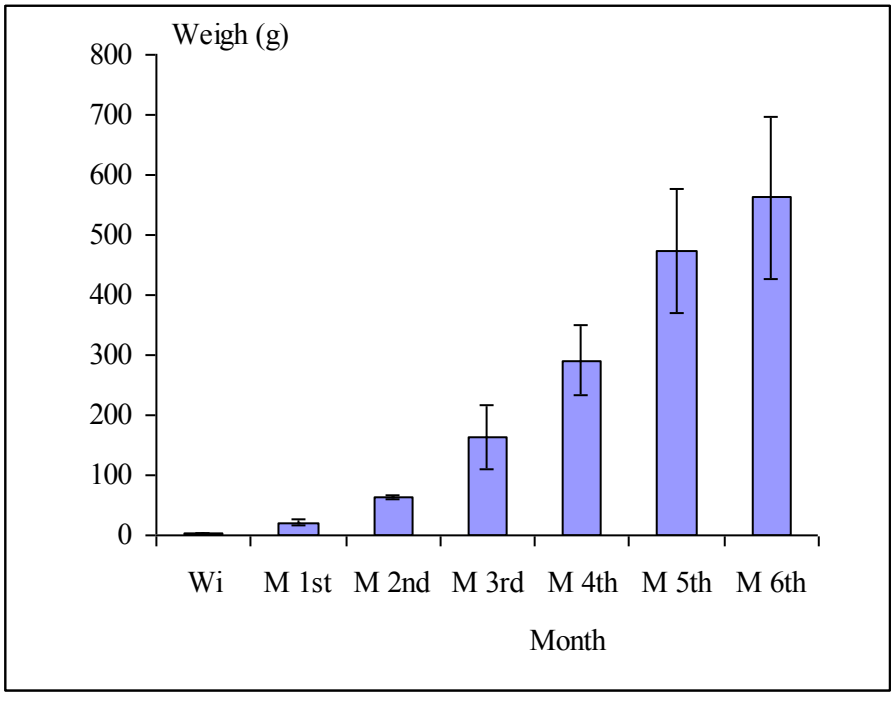


Figure 2.6.1. Growth rate of fish on practical farm demonstrations.

Total cost was 1.61 ± 0.01 USD/kg fish and total income 2.28 ± 0.03 USD/kg fish, farm gate price. Profit was 0.67 ± 0.02 USD/kg fish and profit ratio was 41.7 ± 0.80%.

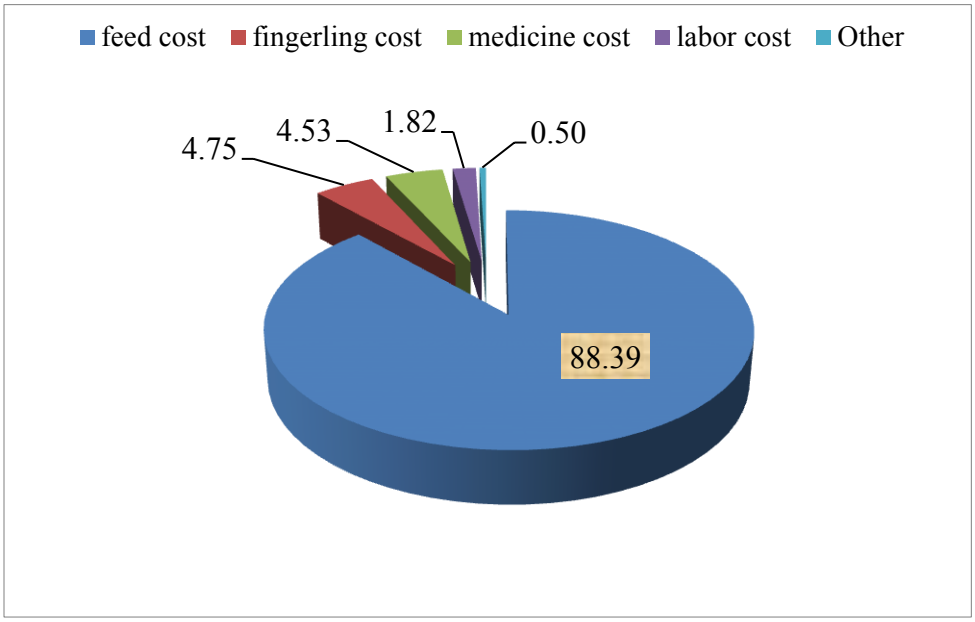


Figure 2.5.2. Division of costs as a percentage of total cost

As in the previous on-farm experiment, the feed cost made up the biggest cost for snakehead culture.

2.7 Study 7: Investigation on the status of commercial pellet usage for cultured snakehead fish (*Channa striata*) in An Giang and Dong Thap provinces

Introduction

In the Mekong Delta, snakehead fish is an economically important culture species which was and is cultured popularly (Nguyen Van Thuong, 2004). There are many snakehead cultured systems commonly in Mekong Delta, including pond culture, hapa culture, cage culture and nylon tank (Le Xuan Sinh and Do Minh Chung, 2010). *Channa striata* and *Channa micropeltes* are the main culture species in Mekong Delta at present. Previous studies were performed (Le Xuan Sinh and Do Minh Chung, 2010; Nguyen Thi Diep Thuy, 2010; Sarowar *et al.*, 2010). These study results illustrated data on the status of culturing snakehead, comprising of household information, culture skills, economic effectiveness and managing on feeding, water quality and disease problems as well. Feeding and feed cost were evaluated as a crucial elements during the culture period. In a study on feed cost, Hien *et al.* (2009) estimated that feed cost play a major role in the total cost of culture systems, contributing 50-80% of total expenditures. Snakehead in the Mekong Delta have been cultured in small scale sytems and "spontaneous systems" (meaning that the farmer did not follow the provincial plan for aquaculture) (Le Xuan Sinh and Do Minh Chung, 2010) using trash fish from both freshwater and marine sources as an important source feed However, rapid development of aquaculture activities resulted in considerable reduction in the availability of trash fish from the wild. Therefore, commercial pellet feed was developed for feeding cultured snakehead. This was considered to be an ideal feed because it reduced culture risks by improving feed and water quality and decreasing the feed cost as well. An investigation on the status of usage of pelleted commercial feed in the culture of snakehead is necessary.

Methodology

Data collecting

Methods of secondary data collecting: secondary data were collected from the reports of local agencies and sectors consisting of Fisheries Stations of An Giang and Dong Thap provinces, Department of Fisheries Resources Management of An Giang and Dong Thap provinces, newspapers and magazines for aquaculture, websites for aquaculture and relating documentations to aquaculture.

Methods of primary data collecting: primary data were collected by directly interviewing 29 farmers in An Giang (An Phu, Long Xuyen and Chau Thanh District) and 12 farmers in Dong Thap (Tam Nong, Tan Hong and Hong Ngu District) by a questionnaireon the farmer's information, culture skills, custody and fish health management and status of disease outbreaks as well as economic effectiveness.

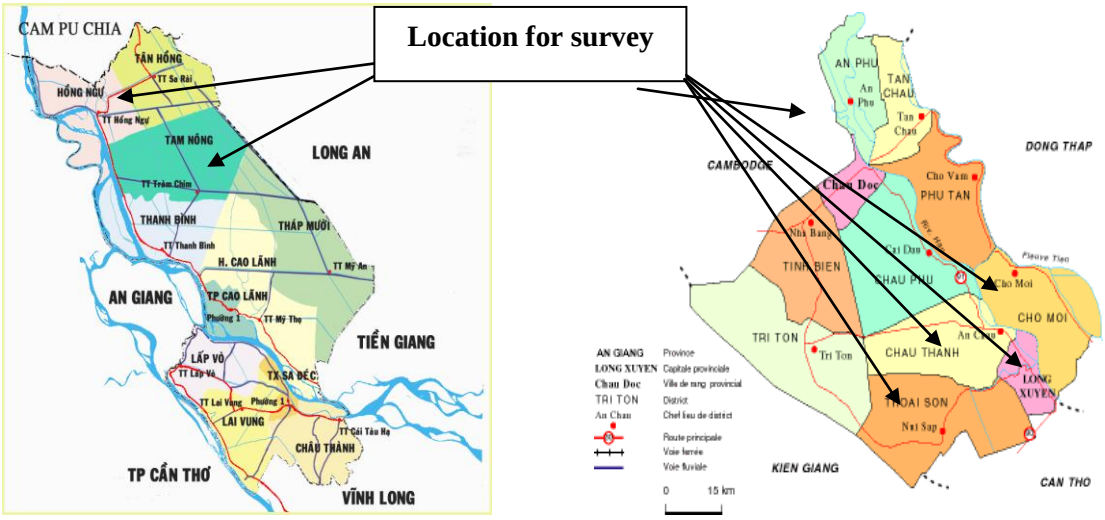


Figure 2.6.1: Location for survey
Figure 2.7.1: Map of study site in An Giang and Dong Thap Province

Statistical analysis

Statistical analysis of collected data was done by SPSS for Windows version 13.0 in descriptive statistic (mean, maximum, minimum, standard deviation) and comparison of means.

Laboratory analysis

The pelleted commercial feeds used in feeding cultured snakehead fish were collected from interviewed farmers to evaluate the chemical composition of feeds which are used for snakehead culture.

The nutrient compositions in the feed such as moisture, crude protein, crude lipid, crude ash were carried out using AOAC (2000) methods. Crude protein (Nx6.25) of feed was determined on total nitrogen determination (Kjeldahl). Crude lipid was defined by Soxhlet method. Crude ash was estimated by burning samples in a furnace overnight at 560^{o}C.

Results

Status of cultured snakehead systems

The investigation results showed that there were 3 systems for culturing snakehead being applied by the households. The highest proportion (85.4%) of farmers culture snakehead inearthen ponds. The plastic covering tank system, which constituted 12.2%, was much less and the lowest percentage (2.4%) of snakehead culture system applied by farmers was hapas in ponds,. In the earthen pond culture system, commercial pellets have been applied because the amount of trash fish is declining. Moreover, snakehead fish farmers are moving away from using trash fish to commercial pellets to practice more intensive culture. However, hapa and plastic-covering snakehead fish culture systems are small scale and farmers can catch trash fish to feed totheir snakehead. The conclusion is that commercial pellets are applied only to the snakehead fish earthen pond culture system.

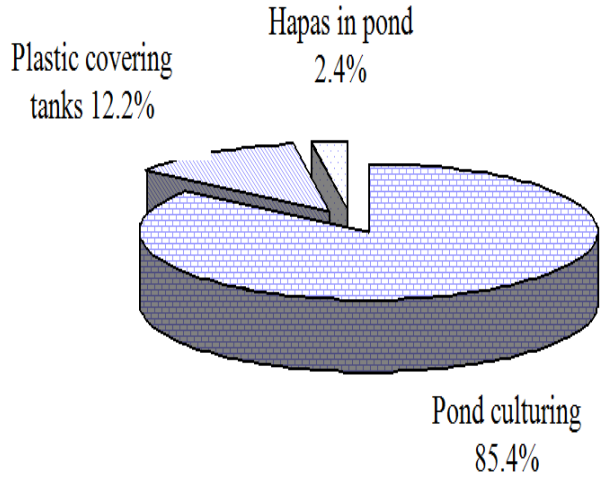


Figure 2.7.2: The types of snakehead fish culture systems in An Giang and Dong Thap Provinces

Snakehead fish culture in earthen pond in An Giang and Dong Thap Provinces

The general information of snakehead fish culture pond in An Giang and Dong Thap Provinces is illustrated in Table 2.7.1. The information of average area of culture pond in this survey illustrated that culturing ponds in Dong Thap Province were larger than in An Giang Province, 1314±741 m^2 in comparison to 941±692 m^2, respectively. Also, the water depth of culture ponds in Dong Thap Province is deeper than in An Giang Province, 3.1±1.1 m and 2.8±0.7 m, respectively. Moreover, stocking density is lower in Dong Thap Province (65.0±43.8 indi/m^2) than in An Giang Province (80.7±85.2 indi/m^2). Overall, there is higher survival rate and total production in Dong Thap Province in comparison to An Giang Province by 6.9% and 21.1 ton, respectively.

Table 2.7.1. Parameters of snakehead fish culture in An Giang and Dong Thap Provinces

Parameters	An Giang province	Dong Thap province
Area (m^2)	940.8±692.2*	1314.2±740.5
Depth (m)	2.8±0.7	3.1±1.1
Stocking density (fish/m^2)	80.7±85.2	65.0±43.8
Price (VND)	265.2±80.3	280.8±50.2
Survival rate (%)	71.0±24.9	77.9±13.0
Total production (ton)	21.8±30.4	42.9±32.6

**average±std*

Information on commercial pelleted feeds used for snakehead fish culture in An Giang and Dong Thap Provinces is shown in Table 2.7.2. The four kinds of feed fed to cultured snakehead fish in pond culture in An Giang and Dong Thap provinces are AquaFeed, Ca Vang, Cargill and UP. However, Cargill was not fed in Dong Thap and the lowest percentage (6.9%) of Cargill was fed in An Giang province. Moreover, feed UP was not fed in An Giang, but this feed was fed commonly in Dong Thap province (75%). The feed for snakehead was a mixture of pellets and trash fish in both provinces (100% of farmers in Dong Thap Province and 93.1% in An Giang Province used a mixture in the first month). In the period of the investigation, a problem, humpback, in the fish was noted, showing the incidence of 4.5 ± 7.0% in An Giang Province and 3.5 ± 5.8% in Dong Thap Province.

Table 2.7.2. Commercial pelleted using for snakehead fish earthen pond culture in An Giang and Dong Thap Provinces

Parameters	An Giang Province	Dong Thap Province
Name of feed		
AquaFeed (%)	20.7	8.3
Ca Vang (%)	72.4	16.7
Cargill (%)	6.9	
UP (%)		75.0
Combined trash-fish & pellet		
No (%)	6.9	
Yes (%)	93.1	100.0
Reasons	**29**	**12**
Fast growth (%)	13.8	16.7
Reducing cost (%)	58.6	50.0
Unavailable of trash-fish (%)	20.7	16.7
Decrease humpback fish (%)	6.9	16.7
Feeding rate (times/day)		
2 (%)	10.3	41.7
3 (%)	86.2	58.3
5 (%)	3.4	
Feeding method		
Direct (%)	96.6	100
Wet form (%)	3.4*	

**Some farmers added water to feed 15 minutes before feeding, because they think the commercial feed is too hard to be palatable to the fish.*

Economic analysis of this investigation on snakehead culture was carried out and demonstrated in Table 2.7.4. The total cost and profit were different in different Provinces. The total cost of culture pond in Dong Thap Province (commercial pellet feed) was lower than in An Giang Province (trash fish feed) by 4,375.8 MVND/ha/crop. Moreover, the result showed higher fixed cost and variable cost in An Giang Province than in Dong Thap Province, which were 44.5 ± 72.5 and 27.0 ± 36.1 and 14,706.6 ± 8,994.1 and 10,348.3 ± 12,792.4 VND/ha/crop, respectively.

Table 2.7.4. Cost and structure of cost of snakehead culture in An Giang and Dong Thap provinces

Parameters	Trash fish feed (N=29)	Commercial pellet feed (N=42)
Total cost (MVND/ha/crop)	14,751.1±9,020.5*	10,375.3±12,803.3
Fix cost (MVND/ha/crop)	44.5±72.5	27.0±36.1
Variable cost (MVND/ha/crop)	14,706.6±8,994.1	10,348.3±12,792.4
Feed (%)	87.8	89.9
Interest (%)	7.1	5.0
Seed (%)	2.5	2.6
Energy (%)	1.5	1.3
Labour (%)	0.7	0.8
Soil sludge out (%)	0.4	0.4

**average±std*

Nutrient composition of feeds fed to cultured snakehead

Seven commercial sources of feed fed to cultured snakehead were investigated. Feed samples were collected from the culture ponds in both survey provinces and were analyzed in the College of Aquaculture and Fisheries, Can Tho University. The results are given in Table 2.7.5. As might be expected, composition of the diets varied markedly. Levels of crude protein ranged from 26.7-44.5% and crude lipid ranged from 1.90-7.49%.

Table 2.7.5. Chemical compositions of feeds

Feed	Moisture (%)	Crude ash (%)	Crude Protein (%)	Crude Lipid (%)	Crude Fibre (%)	NFE
1	6.82	13.8	44.5	2.10	2.95	29.8
2	7.16	12.7	35.7	2.34	3.10	39.0
3	7.56	12.8	43.6	7.49	2.75	25.8
4	9.38	11.9	40.5	6.58	2.84	28.8
5	8.78	14.0	39.0	1.90	4.82	31.5
6	8.97	27.3	42.0	6.50	2.70	12.5
7	10.7	12.8	26.7	6.15	7.02	36.7

Comparison in cultured snakehead fed by commercial pellet feed (CF) and trash fish feed (TF)

We conducted a comparison of operations using trash fish as feed and those using commercial pellet feed. The results of this study are given in Table 2.7.6. The stocking density at farms using CF for feeding cultured snakehead was lower than that at farms using TF (93±94 compared to 110±105 fish/m^2). Survival was 74.8±16.2% for CF, higher than the 59.1±24.9% observed for TF. FCR value in CF (1.4±0.3) was lower than in TF (3.9±0.8). As a result, the return on equity of CF was higher than of TF, showing 0.4±0.7 compared to 0.3±0.3. Therefore, CF system demonstrated an effectiveness in culture snakehead in pond condition.

Table 2.7.6. Comparison between trash fish feed and commercial pellet feed fed cultured snakehead

Parameters	Trash fish feed (N=29)	Commercial pellet feed (N=42)
Area (m^2)	1,345±605*	1,366±1153
Depth (m)	2.9±0.6	3.0±0.8
Stocking density (fish/m^2)	110±105	93±94
Survival rate (%)	59.1±24.9	74.8±16.2
Havested size (g/fish)	743±200	729±215
Total production (ton)	50.7±24.0	39.5±65.1
Yeild (ton/ha/crop)	451±274	293±321
Total of feed (ton/ha/crop)	1,719.8±1,001.5	401.8±498.9
Trash fish (ton/ha/crop)	1,710.8±1,001.5	26.6±32.3
Freshwater trash fish	652.6±981.4	12.2±13.2
Marine trash fish	1,067.1±1,125.6	14.4±19.8
FCR	3.9±0.8	1.4±0.3
Total cost (MVND/ha/crop)	14,751.1±9,020.5*	10,375.3±12,803.3
Fixed cost (MVND/ha/crop)	44.5±72.5	27.0±36.1
Variable cost (MVND/ha/crop)	14,706.6±8,994.1	10,348.3±12,792.4
Income (MVND/ha/crop)	19,250.1±12,907.6	15,762.9±29,250.5
Production cost (1000d/kg)	32.6±6.7	33.8±7.8
Average size (g/fish)	743.1±200.7	728.6±207.6
Price of Selling (1000d/kg)	41.7±5.9	41.2±5.0
Profit (1000d/kg)	7.9±8.2	7.3±
Return on equity	0.3±0.3	0.4±0.7
Loss (%)	20.7	16.7
Profit (%)	79.3	83.3

**average±std*

Pond culture for snakehead iss popular in theMekong Delta, showing the highest proportion (85.4%) of farmers. The lowest percentage was the hapas sytem with 2.4%. The total cost and profit were different in different provinces, being higher in Dong Thap province than in An Giang province. The nutrient composition in feeds fed to cultured snakehead was high in protein and lipid proportion, but very variable. Commercial pellet feed used for feeding cultured snakehead is probably a more sustainable system than using trash fish, based on results for survival, FCR value and return on equity.

CONCLUSION

1. The results from quality analysis showed that fresh-water trash fish exhibited low TVB-N values on the day of capture. Three days later, the fresh-water trash fish was still fresh, whereas the marine trash fish was in stale condition, even though they were still at the distribution site.
2. Giant snakehead start to weaning at 30- 40 day old, replacement rate is 10%/days in 3 days
3. Trial on farm: snakehead fingerlings can be weaned by formulated feed in replacing trash fish in the rate 10%.day^{-1}; the growth of fish and survival rate were not significantly different compared to 100% trash fish use. For giant snakehead, feeding fingerlings with trash fish yielded significantly higher daily weight gain compared to formulated feed ($p<0.05$). However, there was no significant difference between treatments fed different diets in the survival rate ($p>0.05$).
4. The results confirmed that trash fish can be replaced by formulated feed for snakehead culture. Using formulated feed for snakehead culture provided significantly higher profit in AnGiang province than did using trash fish.

5. The snakehead *Channa striata* more effectively used trash-fish from freshwater sources compared to trash-fish from marine sources. Formulated pellet contributed to reduction in the feed cost in snakehead culturing. Moreover, farmers could utilize available local rice bran and freshwater trash-fish through the diet which is 70% freshwater trash-fish and 30% rice bran. In addition, farmers could also use diets MTF+RB+CM 60:20:20 or MTF+RB 50:50 for snakehead culturing. These results will probably be more applicable to farmers in Cambodia than to those in Vietnam.
6. Pond culture in culture snakehead was popular in Mekong Delta, showing the highest proportion (85.4%) of farmers. The lowest percentage was hapas sytem with 2.4%. Pellet feed using for feeding cultured snakehead was probably a more sustainable system than using trash fish based on survival, FCR value and return on equity.

ACKNOWLEDGMENTS

The authors would like to thank Rural Development and Agriculture departments of An Giang and Dong Thap provinces for data collection and snakehead fingerling nursing sites.

LITERATURE CITED

Adron, J. W., and A. M. Mackie, 1978. Studies on the chemical nature of feeding stimulants for rainbow trout, Salmo gairdneri *Richarson. J.* Fish *Bio/.*,4: 303-310.

Ali, A.J., and H.J. Dumont., 2002. "Rice bran as a diet for culturing Streptocephalus proboscideus (Crustacea:Anostraca)." Hydrobiologia 486: 249-254.

AOAC, 2000, Official Methods of Analysis. Association of Official Analytical Chemists Arlington.

Appelbaum, S. and P. Van Damme. 1988. The feasibility of using exclusively arti ficial dry feed for the rearing of Israeli *Clarias gariepinus* (Burchell, 1822) larvae and fry. *J. Appl. Ichthyol*., 4, 105-110

Aquacop, G. Cuzon, R. Chou and J. Fuchs, 1989. Nutrition of the Seabass (*Lates calcarifer*). Advances in tropical aquaculture Tahiti. 9: 757-763.

Baruah, K., A.K. Pal, N.P. Sahu, and D. Debnath, 2007. Microbial Phytase Supplementation in rohu, labeo rohita, a diet enhances growth performance and nutrient digestibility. Journal of the World Aquaculture Society; 38(1): 129-137.

Cao, L., W. Wang, C. Yang, Y. Yang, J. Diana, A.Yakupitiyage, Z. Luo, and D. Li, 2007. Application of microbial phytase in fish feed. Enzyme and Microbial Technology 40: 497– 507.

Cheng, Z.J., and R.W. Hardy, 2004. Effect of microbial phytase supplementation and dosage on apparent digestibility coefficients of nutrients and dry matter in soybean product-based diets for rainbow trout (*Oncorhynchus mykiss*). Journal of the world aquaculture society 35 (1).

Cremer, M.C., Z. Jian and H.P. Lan, 2001. Cage Production of Japanese Sea bass Weaned From Trash Fish to Extruded Feed at Sub-Market Size. Results of ASA/China Feeding Trial 35-01-128.

Cremer, M.C., Z. Jian and H.P. Lan, 2001. Cage Production of Red Drum Weaned From Trash Fish to Extruded Feed at Sub-Market Size. Results of ASA/China Feeding Trial 35-01-127.

Dương Nhật Long, 2004. Giáo trình kỹ thuật nuôi thủy sản nước ngọt

Fermin, A.C., and E.C. Bolivar, 1991. Larval rearing of the Philippine freshwater catfish, *Clarias macrocephalus* (Gunther), fed live zooplankton and artificial diet: a preliminary study. Bamidgeh., 43:87-94.

Goh, Y., and T. Tamura, 1980. Olfactory and gustatory responses to amino acids in two marine teleosts-red sea bream and mulletC. omp. Biochem. Physiol., 66: 217-224.

Hansen, A. C., G. Rosenlund, O. Karlsen, W. Koppe, and G. I. Hemre, 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) - Effects on growth and protein retention. Aquaculture., 272: 599-611.

Hecht, T., and S. Appelbaum, 1987. Note on the growth of Israeli sharptooth catfish (*Clarias gariepinus*) during the primary nursing phase. Aquaculture., 63:195-204.

Hertrampf, J. W., and F. Piedad-Pascual, 2000. Handbook on ingredients for aquaculture feeds. Kluwer academic publishers Dordrecht/ Boston/ London, The Netherlands. 573pp.

Hien, T. T. T., D. T. Yen, and L. B. Ngoc, T. L. C. Tu, H. D. Phuong, and L. S. Heng, 2006. Study on the use of defatted rice-bran in diets for fish culture. Science report in Can Tho University 1: 175-183.

Hien, T. T. T., T. V. Nhi, T. L. C. Tu, and N. T. Phuong, 2006. Evaluation Of Different Ingredient Sources On Culturing Striped Catfish In Cage In An Giang Province - Vietnam (*Pangasius hypothalmus*). Science report in Can Tho University 1: 158-168.

Hien, T. T. T., T.T. Be, C.M. Lee, and D.A. Bengtson, 2009. Replacement of fish meal with soybean meal in diets for snakehead, Channa striata. Paper presented at Word Aqualculture Society, Seattle, Washington, February 15–18, 2009.

Hughes, K.P., and Jr. Soares, 1998. Efficacy of phytase on phosphorus utilization in practical diets fed to striped bass (*Morone saxatilis*). Aquaculture Nutrition; 4(2): 133-140.

Hung, L.T., B.M. Tam, P.Cacot and J.Lazard, 1999. Larval rearing of the Mekong catfish, *Pangasius bocourti* (Pangasidae, Siluroidei): Substitution of Artemia nauplii with live and artificial feed. Aquatic Living Resource, 12(3):229-232

Jackson, L.C, H.Li. Meng, and E. H. Robinson, 1996. Use of Microbial Phytase in Channel Catfish (*Ictalurus punctatus*) Diets to Improve Utilization of Phytate Phosphorus1. Journal of the World Aquaculture Society; 27(3): 309-313.

Kerdchuen, N., and M. Legendre, 1994. Larval rearing of an African catfish, *Heterobranchus longifilis*, (Teleostei, Clariidae): a comparison between natural and artificial diet. Aquatic Living Resource, *7*:247-253.

Lavens, P., and P. Sorgeloos, 1996. Manual on the production and use of live food for Aquaculture. FAO, Fisheries Technical Paper No. 361. Rome. FAO., 295:283-288.

Lê Thị Tươi, 2010. Nghiên cứu sử dụng cám gạo và mì lát làm thức ăn cho cá lóc giống. Luận văn tốt nghiệp đại học. 37 trang.

Lê Vinh Phong, 2009. Khả năng sử dụng bột đậu nành thay thế bột cá trong khẩu phần ăn của cá lóc. Luận văn tốt nghiệp đại học. 23 trang.

Lê Xuân Sinh, 2008. Giáo trình kinh tế thủy sản

Léger, P., D.A. Bengtson, K.L. Simpson, and P. Sorgeloos, 1986. The use and nutritional value of Artemia as a food source. Oceanogr . *Mar. Biol. Ann. Rev,* 24:521-623.

Liti, D. M., R. M. Mugo, J. M. Munguti, and H. Waidbacher, 2006. Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize. wheat and rice) in fertilized ponds. Aquaculture nutrition.,12: 239-245.

Lưu Vương Khang, 2010. Nghiên cứu sử dụng cám gạo thay thế cá tạp làm thức ăn cho cá lóc giống. Luận văn tốt nghiệp đại học. 30 trang.

Mackie, A. M, 1982. Identification of the gustatory feeding stimulants. In: Chemoreception in Fishes, Hara, T.J. (ed.). Elsevier Scientific Publication Co., Amsterdam., 275-291.

Mackie, A. M., and A. I. Mitchell, 1983. Studies on the chemical nature of feeding stimulants for the juvenile European eel, Anguilla anguilla (L.). *J.* Fisi. *Bio*/., 22: 425-430.

Mackie, A.M., and A. I. Mitchell, 1985. Identification of gustatory feeding stimulants for Fish-Applications *in* Aquaculture, Nufrition and Feeding *in* Fisi, 177-1 89.

Martinez J. B., S. Chatzifotis, P. Divanach, and T. Takeuchi, 2004. Effect of dietary taurine supplementation on growth performance and feed selection of sea bass *Dicentrarchus labrax* fry fed with demand-feeders. Fisheries science; 70(1); 74:79.

Mohanta, K. N., S. N. Mohaty, J. K. Jena, and N. P. Sahu, 2006. Apparent protein, lipid and energy digestibility coefficients of some commonly used feed ingredients in formulated pelleted diets for silver barb, *Puntius gonuonotus*. Aquaculture nutrition (12): 211 -218.

Ngô Yến Tuyết, 2009. Khảo sát tình hình nuôi cá lóc thương phẩm ở thành phố Cần Thơ. Luận văn tốt nghiệp đại học. 81 trang

Nguyễn Văn Tiến, 2007. Khảo sát tình hình sử dụng thức ăn trên tôm càng xanh (*Macrobrachium rosenbergii*) và cá lóc (*Ophiocephalus striatus*) ở An Giang, Đồng Tháp và Cần Thơ. Luận văn tốt nghiệp đại học. 70 trang

Nutrient reasearch Council (NRC). Nutrient requirements of fish. Washington, DC: National Acedemiy Press; 1993, 71pp.

Perschbacher, P., and R. Lochmann, 1999. Effect of feeding Pelleted versus non-pelleted defatted rice bran on Nile Tilapia *Oreocgromis niloticus* Production and Water quality in ponds. Asian Fisheries science., 12: 49-55.

Phan Hồng Cương, 2009. Tình hình sử dụng cá tạp và khả năng sử dụng bột đậu nành trong phối chế thức ăn chế biến nuôi cá lóc. Luận văn tốt nghiệp cao học. 69 trang.

Phan Văn Dương, 2010. Nghiên cứu sử dụng cám gạo làm thức ăn chế biến cho cá lóc. Luận văn tốt nghiệp đại học. 18 trang.

Qin, J., A.W.F., 1996. Size and feed dependent cannibalism with juvenile snakehead *Channa striatus*. Aquculture 4, 313-320.

Rachmansyah, U., Palinggi, N.N. and Williams, K., 2009. Formulated feed for tiger grouper grow-out, AquacultureAsia Magazine, pp. 30-35.

Ronyai, A., and A. Ruttkay1990. Growth and food utilization of wels fry (*Silurus glanis*) fed with tubifex. Aquacult. Hung. (Szarvas) VI, 193– 202.

Sajjadi, M., and C.G. Carter, 2004. Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar*, L.). Aquaculture Nutrition; 10(2),: 135-142.

Soltan, M.A., M.A. Hanafy, and M.I.A. Wafa, 2008. Effect of replacing fish meal by a mixture of different plant protein sources in Nile tilapia (*Oreochromis niloticus* L.) diets. Global Veterinaria. 2: 157-164.

Sverdrup-Jensen, S. Fisheries in the Lower Mekong Basin: Status and Perspectives. MRC Technical Paper No. 6. Mekong River Commission, Phnom Penh. 103 pp.

Takeda, M., K. Takii, and K. Matsui, 1983. Identification of feeding stimulants for juvenile eel. *Bull.Jap. Soc. Scient.* Fisi., 59: 645-651.

Trần Thị Thanh Hiền & Nguyễn Anh Tuấn, 2009. Dinh dưỡng và thức ăn thủy sản

Trieu, N. V., D. N. Long, and L. M. Lan, 2001. Effects of Dietary Protein Levels on the Growth and Survival Rate of Snakehead (*Channa striatus*, Bloch) Fingerling. Development of new technologies and their practice for sustainable farming in Mekong Delta, Cuu Long rice research institute Omon, Cantho, Vietnam.

Trương Thủ Khoa và Trần Thị Thu Hương, 1993. Định loại cá nước ngọt vùng đồng bằng sông Cửu Long

Wang, Y., K. Li, H. Han, Z. X. Zheng, and D. P. Bureau, 2008. Potential of using a blend of rendered animal protein ingredients to replace fish meal in practical diets for malabar grouper (*Epinephelus malabricus*). Aquaculture., 281: 113–117.

Assessment of Integrated Pond-Cage System for the Production of Nile Tilapia for Improved Livelihood of Small-Scale Fish Farmers in Kenya

Sustainable Feed Technology/Experiment/09SFT02PU

Kwamena Quagrainie
Purdue University
West Layfayette, Indiana, USA

Charles C. Ngugi
Kenyatta University
Nairobi, Kenya

Sammy Macharia
Ministry of Fisheries Development
Nairobi, Kenya

Kuria Gladys Mwihaki
Moi University
Eldoret, Kenya

ABSTRACT

The study analyzed the effects of three different stocking densities of tilapia in cages on growth and yield in an integrated cage-pond system and compared alternate feeding regimens on growth and yield performance under the three different stocking densities. The costs of producing tilapia at the different stocking densities and feeding regimens in the integrated system were also compared. The results from this study indicate that stocking density considerably influences the growth performance, survival, yield and economic performance of *O. niloticus* in cages under a cage-cum-pond culture system. The stocking density of 50 fish m^{-3} gave the best growth, survival and economic benefits but the lowest yield. This suggests that a cage-cum-pond culture system of *O. niloticus* with stocking density of 50 fish m^{-3} for larger size of fish in a short period and 100 fish m^{-3} provide the highest production. Integration of cage and pond culture therefore increases yield and thus farmers stand a chance of benefiting from two crops in the same facility. It is recommended that the appropriate stocking density in a cage-cum-pond culture system for larger fish in a short period of time should be 50 fish m^{-3}. On the other hand, for higher production, stocking cages at 100 fish m^{-3} is recommended.

INTRODUCTION

In the recent past, integrated cage-cum- ponds culture system has been developed and practiced using combination of catfish-tilapia and tilapia-tilapia (Yi et al., 1996 and Yi, 1997a, Yi 1997b). Integrated cage-cum- ponds culture is a system in which fish are fed with artificial diets in cages suspended in ponds, while same species or others low value fish are stocked in open pond water to utilize natural foods derived from cage wastes. The technique uses the niche optimization concept for feeding; the fish in cages are fed while those in open waters are either fed at lower rates or not fed at all. Pond fish, therefore, derive their nutrients from uneaten foods from the cages or from autotrophic and heterotrophic food chains (Yi et al., 1996). The aim is to rear fish in a cage while the pond fish utilize the uneaten cage feeds and the plankton generated in the pond to satisfy the bio-energetic needs. In such practices, the nutrient utilization efficiency could reach more than 50%, compared to about 30% in most intensive culture systems (Coche 1979). To limit competition for food, raise pond carrying capacity, increase pond fish

production, higher supply of artificial feed is required. This systems, provides small-scale farmers an opportunity to use their limited resources to increase fish yield, generate more income.

Rural pond culture in Kenya is moving from subsistence to small-scale commercial culture of fish. The aquaculture industry is transitioning from the rural subsistence enterprise to commercial profit-oriented aquaculture business. Small-scale commercial farmers are utilizing improved management practices such as stocking densities, feeding regimens, and feed nutrient to enhance their economic returns (Quagrainie et al., 2009). The current major production system is pond culture with an average size of 400m^2 stocked with Nile tilapia (Oreocrhomis niloticus) and the African catfish (Clarias gariepinus).

Feeding issues appear to be one of the challenges facing Kenyan small-scale commercial farmers. Studies in Southeast Asia have suggested improvements in growth and yields of Nile Tilapia in integrated cage-cum-pond systems (Yi and Lin, 2001; Yi, Lin and Diana, 1996). The integrated system allows the open pond water to utilize cage wastes as fertilizers, generating natural food in the pond. The integrated system is environmentally friendly because less waste nutrients are released to the public water systems. Profitability from such venture is highly dependent on fish performance in the cage and static ponds. Yi and Lin (2001) concluded that an appropriate integrated cage-cum-pond system depends on appropriate stocking densities for tilapia rotation culture. The underlying hypothesis is that greater amount of wastes from increased biomass in the cages would enhance the productivity in the open ponds. The specific objectives of this study are:

1. To analyze the effects of three different stocking densities of tilapia in cages on growth and yield in an integrated cage-pond system
2. To compare alternate feeding regimens on growth and yield performance under the three different stocking densities.
3. To compare the costs of producing tilapia at the different stocking densities and feeding regimens in the integrated system
4. Conduct on-farm trials to test integrated cage-pond system technologies developed in this study and evaluate costs and benefits to local fish farmers through on-farm trials

Hypotheses

1. H_0: Different stocking densities have no effect on growth of *O. niloticus* in cages suspended in static ponds in an integrated cage-cum-pond culture system.
2. H_0: Different stocking densities have no effect on survival rates of *O. niloticus* in cages suspended in static ponds in an integrated cage-cum-pond culture system.
3. H_0: Different stocking densities have no effect on yield of *O. niloticus* in cages suspended in static ponds in an integrated cage-cum-pond culture system.
4. H_0: Integrated cage-cum-pond culture system has no effect on the growth and yield of *O. niloticus* in the pond.
5. H_0: Different stocking densities have no effect on economic benefits of *O. niloticus* in cages suspended in static ponds in an integrated cage-cum-pond culture system.

METHODOLOGY

Study Area

The study was conducted at Mwea Aquafish Farm (MAF). The warm water fish farm is located 110km North East of Nairobi on the Nairobi-Embu highway and 1.5km from Kimbimbi town. The study area lies at 0˚36.73'S, 37˚22.84'E and 1208m above the sea level. Daily temperatures range from 16.9˚C to

29˚C with cool seasons ranging between 16.9 - 23˚C and warm seasons between 19 - 29˚C. Rainfall ranges between 18.5mm – 223mm in rainy months and 0mm in dry months.

Experimental Design

The study was done in a 1300m^2 earthen pond using 9 cages each with a volume of 1 m^3. The cages had a frame made from 2-inch diameter PVC pipes covered with a 1-inch netting material. The tubing of the upper frame was completely sealed to offer a self-floating mechanism, while the lower frame was perforated to allow it to sink once water filled the tubing. Floating feed rings with a diameter of 50cm made from flexible 15mm PVC tubing were installed to minimize the amount of floating feeds that would slip out of the sides of the cage during feeding.

Hand sexed male *O. niloticus* fingerlings (Mean weight 65.57g ± 0.766; Total length 15.74cm ± 0.076) and *O. niloticus* fry from the M.A.F. hatchery were stocked in the cages and the open pond water respectively (the pond was stocked six weeks after stocking of cages). Fish were reared for a period of 180 days with close monitoring and sampling between 2nd September, 2010 and 2nd March, 2011. Cages were randomly allotted three treatments and stocked at varying densities of 50, 75 and 100 fish per m^3. Each treatment had three replicates to increase data accuracy and reduce cases of bias. Fish were fed with commercial floating feeds.

Feeding was done through hand feeding to caged fish at 4% body weight for the first month and reduced as the fish grew to 1.5 in the sixth month. Fish were fed twice a day at 10.00 hrs and 16.00 hrs. Fish in the experimental pond were not fed with the commercial diet but browsed on the plankton material in the pond; however, they were allowed to feed on feeds that slipped from the sides of the cages. Feeds were adjusted every month based on calculated biomass.

The experimental pond was stocked with 4m^{-2} *O. niloticus* as is the practice by small scale commercial fish farmers for static pond culture in this region. Prior to stocking, the pond was fertilized with 20kgN ha^{-1} wk^{-1} and 5kg P ha^{-1} wk^{-1} using Urea and Di-ammonium phosphate (DAP); which is a standard procedure. After 30 days, the rate of Urea application was lowered to 10kgN ha^{-1} wk^{-1} as measures to correct ammonia build up in the pond. Due to evaporation and pond seepage, the pond was topped up every two weeks to maintain the water level at 1.3m.

Sampling

Dip and seine nets were used to sample fish in cages and in the pond respectively. 30 fish from every cage and 100 from the open pond were sampled during each sampling occasion. On completion of the experiment, all fish were harvested from both the cages and the experimental pond. Their mean weights were recorded and their yields computed. Plankton net was used to sample plankton in the pond. Stomach content analysis of fish (both from the cages and from the pond) was also done.

At the end of the experiment, fish were harvested using repeated netting, and total yield determined. Minitab statistical software was used for analysis. The effects of the three stocking densities on growth, survival, yield performance and economic benefits were analysed using Ordinal Logistic Regression with time in months as the response variable and the different stocking densities and the factors. ANOVA was also used to test significance differences among the treatments. The following parameters were calculated as indicated. Mortalities were recorded as they occurred. Their numbers were also determined by counting the remaining fish in the cages at each sampling date.

A full enterprise budgeting analysis was used to compare the relative profitability of the different stocking densities by estimating all income and expenditures of each treatment.

Dissolved oxygen (DO), temperature and pH were measured *in situ* twice a week. DO and temperature were measured using DO meter (model: YSI 550A) while pH was measured using a pH meter (model: HI 98127). Water samples were also collected monthly in the morning and afternoon from the pond between 0800 and 1800 h for analysis of nitrite-nitrogen (NO_2-N), total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP). All the analyses were conducted following the standard analytical procedures detailed in Boyd and Tucker (1992).

RESULTS

Fish Growth

The overall growth performance attributes of *O. niloticus* in cages are presented in Table 2. The stocking weights of *O. niloticus* among the treatments were statistically similar (p=0.992). The same case applied for the stocking weights (p=0.995). After the study period of 180 days, the largest fish in cages weighed 590g recorded in fish stocked at 50 fish m^{-3} as shown in Table 2 while the smallest fish was found in the treatment with the highest stocking density of 100 fish m^{-3} weighing 180g. Fish reared at the lowest stocking density were more uniform in sizes during harvest; however, fish reared at the highest stocking density were less uniform despite being stocked at the same size.

The SGR of fish stocked at 50 fish m^{-3} was higher than that of fish stocked at 75 fish m^{-3} as well as those stocked at 100 fish m^{-3} which showed the lowest SGR. The condition factor of fish stocked at 50 fish m^{-3} was higher than observed in the other two treatments with fish stocked at 100 fish m^{-3} having the least condition factor. Fish stocked at 100 fish m^{-3} had the highest total yield as compared to the other two treatments.

Mean length of fish stocked at 50 fish m^{-3} was higher as compared to the other two treatments. Fish stocked at 100 fish m^{-3} had the least length as shown in figure 1. The same was observed for the mean weight. Both the lengths and the weights showed a statistical significant difference (p=0.000).

There was a significance difference (p = 0.000) in the growth of fish among the different treatments. Both the weights and the lengths showed this difference. As shown in figure 2, fish stocked at 50 fish m^{-3} had the best growth as compared to the other two treatments. After the first month, the lengths of *O. niloticus* among the treatments showed no significance difference (p=0.434) as did the weights (p=0.854). At the second month, the length of fish stocked at 50 fish m^{-3} showed statistical difference (p=0.000) from the rest, however, the lengths of fish stocked at 75 fish m^{-3} and 100 fish m^{-3} still remained statistically similar (p=0.833). The same was observed in weights between fish stocked at 75 fish m^{-3} and 100 fish m^{-3} (p=0.212). At the third month, all treatments showed statistical difference (p=0.000) which was maintained to the end of the experiment.

Table 1: A summary of the various experimental parameters (Mean ± SE)

Growth parameter	50 fish m^{-3}	75 fish m^{-3}	100 fish m^{-3}
Stocking Length (cm)	15.72 ± 0.12^a	15.74 ± 0.13^a	15.74 ± 0.15^a
Stocking Weight (g)	65.47 ± 1.07^a	65.59 ± 1.24^a	65.67 ± 1.65^a
Mean Harvest Length (cm)	25.57 ± 0.137^a	24.03 ± 0.139^b	23.15 ± 0.156^c
Mean Harvest Weight (g)	360.00 ± 7.34^a	281.28 ± 4.73^b	247.94 ± 4.64^c
Harvest maximum Length (cm)	30.0	26.8	26.4
Harvest minimum Length (cm)	23.0	21.1	20.3
Harvest maximum Weight (g)	590.0	390.0	350.0
Harvest minimum Weight (g)	260.0	200.0	180.0

Growth parameter	50 fish m^{-3}	75 fish m^{-3}	100 fish m^{-3}
Mean Yield (kg)	17.35^{a}	18.58^{a}	20.91^{b}
SGR (% day^{-1})	0.41	0.35	0.32
FCR	1.9^{a}	2.6^{b}	3.1^{c}
Condition factor	2.15^{a}	2.03^{b}	2.00^{c}
Survival (%)	96.67^{a}	89.78^{b}	83.00^{c}

NB: For every parameter and treatment, means with different letters as superscripts are statistically different.

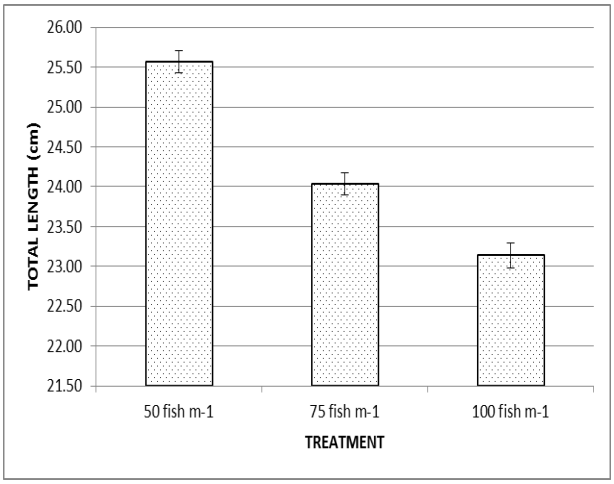


Figure 1: Total Length (Mean ± SE) for each treatment

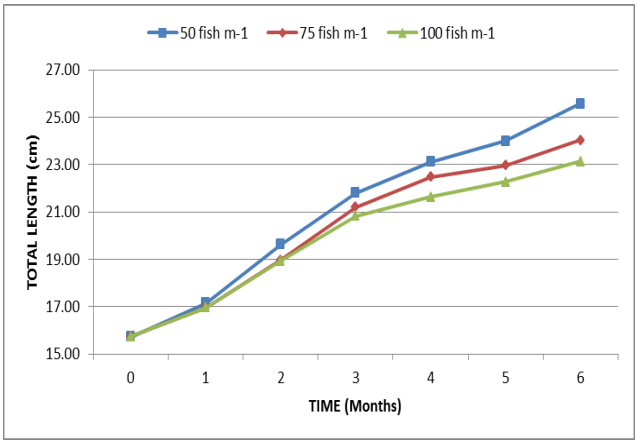


Figure 2: Total Length of fish over a period of 6 months for the three treatments.

The SGR of fish in all treatments had a continued decline. Reduction in the SGR was highest in fish stocked at 100 fish m^{-3} and lowest in fish stocked at 50 fish m^{-3} with fish stocked at 75 fish m^{-3} remaining as the intermediate. These trends were maintained throughout the experimental period. The SGR corresponded to cage biomass such that, as the cage biomass increased, SGR continually declined towards stagnation.

At a stocking density of 50 fish m^{-3}, the general equation of Log W versus Log L indicated a slope (b) value of 3.3191; a slope of 2.7734 for the fish stocked at 75 fish m^{-3}; and 2.6689 for fish stocked at 100 fish m^{-3}. This shows a decrease in the slope as the stocking density increased. The lowest stocking density of 50 fish m^{-3} had the highest condition factor (K) as compared to fish stocked at 75 and 100 fish m^{-3}. The fish stocked at 100 fish m^{-3} had the lowest condition factor.

Food Conversion Ratio of *O. niloticus* stocked in cages suspended in a pond decreased with decrease in stocking density. Fish stocked at 100 fish m^{-3} had the highest FCR of 3.1 followed by fish stocked at 75 fish m^{-3} with FCR of 2.6 and finally fish stocked at 50 fish m^{-3} had the least FCR of 1.9 as shown in figure 3. FCR among the three different treatment was statistically different (P= 0.000).

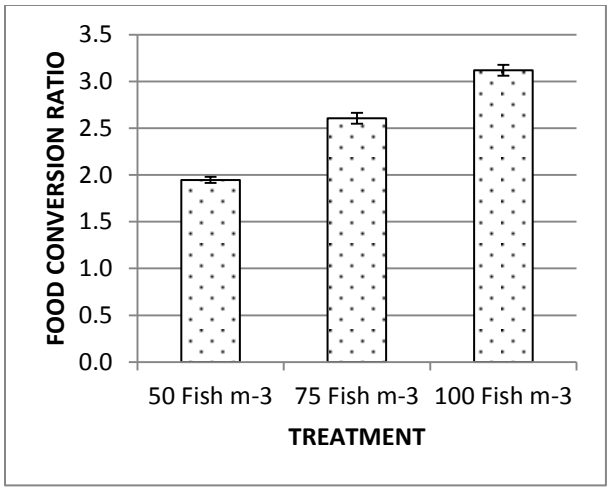


Figure 3: FCR of *O. niloticus* reared in cages suspended in a pond under 3 different stocking densities.

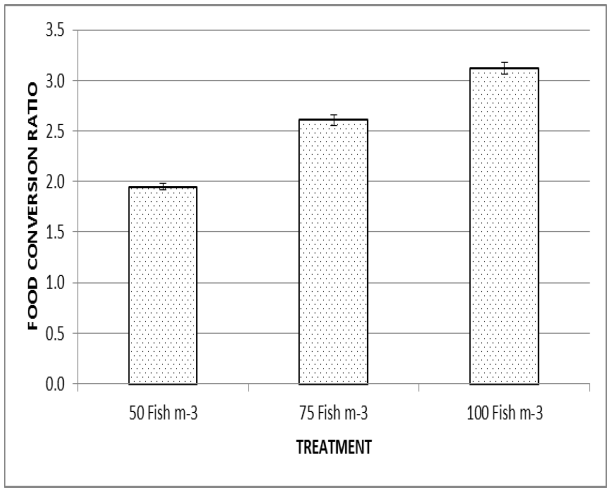


Figure 4: Percentage Survival of *O. niloticus* in cages under three different stocking densities

Fish Survival

Percentage survival of *O. niloticus* stocked in cages suspended in a static-water pond increased with a decrease in the stocking density. Fish stocked at 50 fish m^{-3} had the highest survival rate of 96.67% followed by fish stocked at 75 fish m^{-3} at 89.78% and finally 100 fish m^{-3} with the least survival rate at 83.00% as shown in figure 7. Mortalities were first experienced on the fifth day when feeding started and remained high for the first two weeks before the fish acclimatised to the new environment. Mortalities were also experienced after every sampling occasion.

Yield

Yields of fish increased with increase in the stocking density. The highest stocking density (100 fish m^{-3}) yielded higher than treatments where fish were stocked at 50 and 75 fish m^{-3}. Fish stocked at 50 fish m^{-3} had the lowest yield as shown in figure 5. Mean yield of fish stocked at 100 fish m^{-3} had a significance difference (p=0.000) from the other two treatment. Mean yields of fish stocked at 50 fish m^{-3} and 75 fish m^{-3} showed no significance difference (p=0.050).

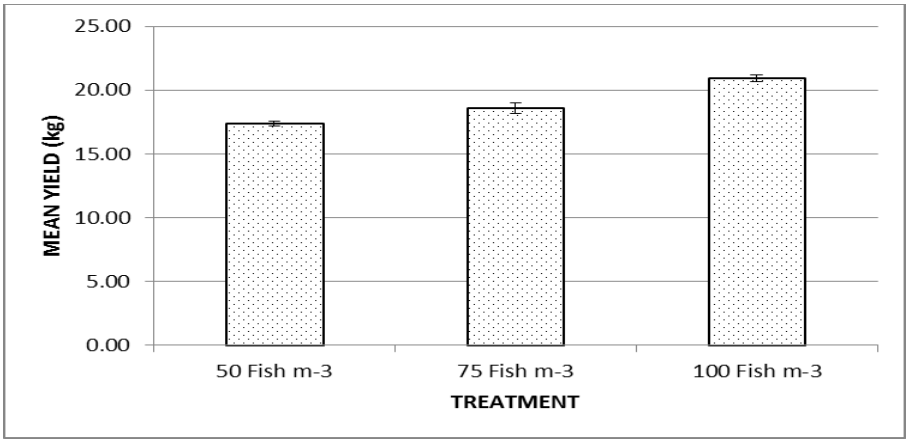


Figure 5: Mean yield of *O. niloticus* stocked in cages at different stocking densities.

Pond Fish

Fish in ponds ranged in their weights between 1g and 190.0 g. They showed division in five classes which included fish between below 6g (fry – 28,500); 6.6 – 38.7g (3751 pieces); 43.9 – 69.4 g (1386 pieces); 71.70 – 98.9g (342 pieces); and 103.9g – 190.0g (151 pieces) as shown in table 2.

Plankton Composition

Both the phytoplankton and zooplankton were available in the pond water where the cages were suspended. Phytoplankton dominated with *Volvox spp* (60%) as the dominant species found. Others include: *Scenedesmus, Pandorina, Aphanocapsa, Tetraedron, Euglena spirogyra.* Zooplankton were dominated by *Daphnia spp* (55%); others include: *Flagaria capucina, Nauplius*

Table 2: A table showing the various classes of fish harvested in the pond

	Minimum		Maximum		Mean		
Class	TL (cm)	WT (g)	TL (cm)	WT (g)	TL (cm)	WT (g)	Yield (kg)
6 - 40g	7.00	6.60	13.30	38.70	10.32	21.47	80.53
40-70g	13.10	43.90	16.20	69.40	14.63	55.47	76.88
70 – 100 g	15.50	71.70	19.00	98.90	16.79	87.73	30.00
> 100g	18.5	103.90	22.30	190.00	19.47	129.31	19.53

Stomach Content Analysis

Analysis of the stomach content of fish in cages for the three different stocking densities (i.e. 50, 75 & 100 fish m^{-3}) showed that fish in cages, highly depended on the commercial feed given to them with percentages of 95%, 93% and 93% respectively. On the other hand, fish in the open pond, consumed 88% plankton material and 12% commercial feed.

Table 3: Mean, Min and Max water quality parameters measured during the study period.

PARAMETER	MEAN ± SE	MINIMUM	MAXIMUM
Dawn DO (mg l^{-1})	3.023 ± 0.0496	1.9900	3.5600
Evening DO (mg l^{-1})	6.827 ± 0.0998	5.2200	8.4400
Dawn pH	8.279 ± 0.0232	7.9000	8.7000
Evening pH	8.815 ± 0.0222	8.4000	9.3000
Dawn Temperature (^oC)	22.750 ± 0.111	21.100	24.300
Evening Temperature (^oC)	26.798 ± 0.111	25.000	28.800
Total phosphorous (mg l^{-1})	0.085 ± 0.0034	0.0800	0.1000
SRP (mg l^{-1})	0.036 ± 0.0008	0.0330	0.0390
Total nitrogen (mg l^{-1})	0.287 ± 0.0061	0.2700	0.3100
NO_2-N (mg l^{-1})	0.009 ± 0.0004	0.0080	0.0110

Water Quality Parameters

The water quality parameters in the pond where the cages were suspended remained within the tolerable ranges for the optimal growth of *O. niloticus* as shown in table 3.

Economic Benefits

Returns decreased with increase in stocking density. The highest stocking density (100 fish m^{-3}) had the lowest returns as compared to the other two treatments where fish were stocked at 50 and 75 fish m^{-3}. Fish stocked at 50 fish m^{-3} had the highest returns as shown in figure 6. Mean returns of fish stocked at 50 fish m^{-3} had a significance difference (p=0.003) from the other two treatment. However, mean returns of fish stocked at 75 fish m^{-3} and 100 fish m^{-3} showed no significance difference (p= 0.735). Fish from the pond

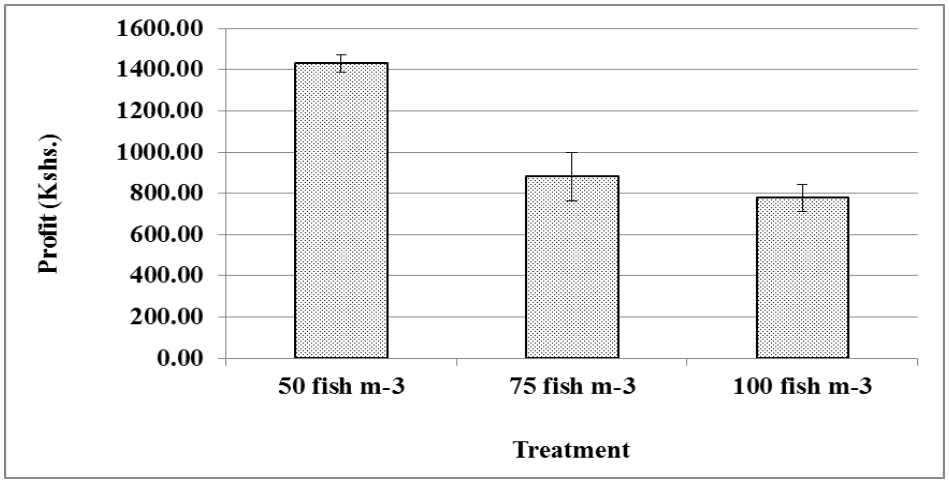


Figure 6: Mean returns accrued from the different stocking densities

were sold at different prices depending on their size. Fish above 120g were sold as broodstock. Individual cost and revenue are shown in Tables 4. Enterprise budget for *O. niloticus* stocked at 100 fish m^{-3}shown in Table 5.

On-Farm Trials

Preparatory contacts with farmers in Central Province – Kenya began immediately after the research work on cage-cum-pond culture started. Three groups of farmers were identified i.e. Ruiru Youth for Development and Environment Conservation group – Thika; Rugita Youth Development group – Kikuyu; and Karunda Whiteland Youth Development Group – Nyeri. A workshop to introduce the farmers to cage culture was held on 16th October, 2010 at Mwea Aquafish Farm. Farmers were taught the importance of cage culture, cage construction, site selection for placement of the cages, feeding, record keeping, and stocking densities among other cage management practices. They were also introduced to pond management practices. In addition to the farmers who participated in the workshop, extension officers, AquaFish CRSP personnel, Mwea Aquafish Farm staff and a student involved in the cage culture research work were involved. A more intensive training was conducted from 23rd November to 5th December, 2010 at Mwea Aquafish Farm where two farmers from each group attended. During this training, the farmers constructed cage frames and also learnt how to make a complete cage. After the training, farmers were given materials for cage construction which they started from 6th December, 2010 at their respective sites. Each group constructed six cages.

On-farm trials of cages in ponds and of cages in dams were conducted by the group members between December 2010 and July 2011. A post-trial workshop was held on 8th July, 2011 at MAF. The three groups of farmers participated in the workshop, along with extension officers who have been working with the various groups, AquaFish CRSP personnel, MAF staff, representatives from the Ministry of Youth Affairs, and a student involved in the cage culture research work.
Each group made a presentation of how they have progressed in cage farming, their experiences in fish farming and the challenges they are facing. Farmers were given a chance to ask questions and discuss the impacts they have had on the community around them.

Challenges faced

Twiga dam

- High fish mortalities
- Theft of fish
- Accessibility of the cages – inefficient boat
- Uncommitted members
- Poor record keeping

Gathathi-ini dam

- Accessibility of the cages – inefficient boat (heavy)
- Uncommitted members
- Insufficient funds
- Destruction of hapa nets by predators (leading to loss of fish)
- High water turbidity

Rungiri dam

- Uncommitted members
- Water resource conflict
- Poor security
- Drug trafficking by the community within the vicinity of the dam
- Crime (Murder and suicide) – dumping of murdered people in the dam
- Leadership problem (which is now resolved)

DISCUSSION

Fish Growth

Growth trends of fish in this study indicated that there was uniform growth pattern in length and weight of fish stocked at the three different densities for the first month of the rearing period. This could be attributed to the fact that fish transferred to a new environment take time to acclimatize. This condition, however, changed in the subsequent months based on the different stocking densities. The growth performance of tilapia is significantly related with the stocking density of the fish (Chakraborty *et al.,* 2010).

In this study, the growth of *O. niloticus* in cages was found to be density dependent with increased stocking density having a negative effect on final mean body weight, final mean total length, SGR, FCR and condition factor. Similar observations were made by Yi and Lin (2001), where increased fish biomass of Nile tilapia in cages has a significant negative effect on the final mean body weight. Increased rearing density negatively affected the mean body weight, final mean total length, SGR and weight gain of *O. niloticus* fry reared in glass tanks (Opiyo, 2010). This is in agreement with previous studies by: Yi *et al*.,

(1996); Gibtan *et al*., (2008); Mokoro, (2008). However, some researchers have found out a positive effect on growth of fish with increased stocking densities (Baker and Ayles, 1990; Petit *et al.,* 2001).

Table 4: Enterprise budget for *O. niloticus* in a cage-cum-pond culture system

Item	Number	Unit	Cost / unit (Kshs.)	Total cost (Kshs.)	Total cost (US$)
Revenue					
Cage fish	170.52	kg	300	51156	639.45
Pond fish 7 - 40g	3751	piece	15	56265	703.31
Pond fish 40 – 70g	1386	piece	40	55440	693.00
Pond fish 70 - 100g	342	piece	80	27360	342.00
Pond fish > 100g	151	piece	200	30200	377.50
Fry	28500	piece	7	199500	2493.75
Total revenue				**419921**	**5249.01**
Costs					
Variable costs					
Fingerlings	675	piece	15	10125	126.56
Fry	5200	piece	5	26000	325.00
Feed	322.96	kg	60	19377.41	242.22
Labor	23	man/8hr	200	4600	57.50
Fertilizer					
Urea	10.4	kg	50	520	6.50
DAP	2.6	kg	60	156	1.95
Fuel	23	Litres	100	2300	28.75
Total variable costs (TVC)				**63078.41**	**788.48**
Fixed costs					
Cage rent	9	Piece	750	6750	84.38
Pond rent	6	Month	500	3000	37.50
Total fixed costs				**9750**	**121.88**
Total costs (TC)				**72828.41**	**910.36**
Net returns above TVC				356842.59	4460.53
Net returns above TC				347092.59	4338.66

The observed final mean body weight and final mean total length in this study were poor in the high stocking density of 100 fish m^{-3}. The lower growth performance of tilapia at higher stocking density could have been caused by voluntary appetite suppression, more expenditure of energy because of intense antagonistic behavioural interaction (Chakraborty *et al.,* 2010), competition for food and living space (Diana *et al*., 2004) and permanent stress caused by crowding (Ellis *et al*., 2002). Boujard *et al*., (2002) also reported that appetite of fish can be impaired by increase in density. High stocking densities impair visual location of food by making it difficult for fish to follow a trajectory towards the food pellets (Silva *et al.,* 2000) and might have prevented the physical access of pellets by the fish stocked at 100 fish m^{-3}. Poor feeding might also explain the poor performance of fish stocked at high density.

Table 5: Enterprise budget for *O. niloticus* stocked at 100 fish m^{-3}

Item	Quantity	Unit	Unit Cost (Kshs.)	Total cost (Kshs.)	Total cost (US $)
Revenue					
Cage Fish	20.91	kg	300	6272.00	78.40
Total revenue				6272.00	78.40
Costs					
Variable costs					
Fingerlings	100	piece	15	1500.00	18.75
Feed	43.64	kg	60	2618.36	32.73
Labor				383.33	4.79
Fertilizer				16.00	0.20
Fuel				144.44	1.81
Total variable costs (TVC)		Kshs.		4662.13	58.28
Fixed costs					
Cage rent				750.00	9.38
Pond rent				83.33	1.04
Total fixed costs		Kshs./US $		833.33	10.42
Total costs (TC)		Kshs./US $		5495.46	68.69
Net returns above TVC		Kshs./US $		1609.87	20.12
Net returns above TC		Kshs./US $		776.54	9.71
Breakeven price (BEP)		Kshs./US $		155.29	1.94
Breakeven yield (BEY)		kg		10.82	

The fish attained the highest length and achieved maximum body weight when reared at 50 fish m^{-3}. This could be due to the fewer fish per unit space (Sahoo *et al*., 2004) in the cages. Similar results were reported by Gibtan *et al.,* (2008) who recommended 50 fish m^{-3} as the optimal stocking density of *O. niloticus* in cages in Lake Kuriftu in Ethiopia. The average weight for the low stocking density at harvest was 360g. The weights obtained in this study confirm the results obtained in experiments conducted at IAAS which showed that intensive culture of Nile tilapia in cage within pond with feeding can efficiently produce large fish (250-300 g) (Shrestha, 2002). In spite of rearing the fish in cages for 180 days, they did not attain an average weight of 500g which was reported by Yi *et al.,* (1996) within a period of 90 days. This could have resulted from poor quality feeds as the diet used in this study had 17% crude protein which was lower than the recommended 28 - 32% CP for Tilapia above 25g (McGinty and Rakocy, 1989; Ofori *et al.,* 2009).

Fish stocked at a high density were less uniform in sizes during harvest despite being stocked at the same size. Among other factors the food accessibility is one of the most difficult parameter to set identical for each fish when density increases (Boujard *et al*., 2002). This could probably be the reason for the varied sizes in fish stocked at a high density of 100 fish m^{-3}.

Specific growth rate of *O. niloticus* in cages under the current study was density dependent with low stocking density performing better than the other two treatments. The trend in this parameter over the

180-day period showed reduction with increase in biomass after the second month. However, despite this reduction, low stocking density maintained the highest SGR throughout the study. The high SGR observed in low stocking density could be attributed to by better accessibility of feeds due to adequate space.

The regression coefficient (r) for length-weight relationship of *O. niloticus* in cages for the three different stocking densities was high (above 80%). This indicates that the weight of fish increases with increase in length. The length-weight relationship within a species differs according to the robustness of individual fish, however, similar findings have been reported in previous studies on different fish species by: Layèyè (2006), Ayoade and Ikulala (2007); Ndimele *et al*., (2010). The condition factor for *O. niloticus* in cages was greater than 1 for all the treatments and this shows that all fish were above average condition. The low stocking density had the highest condition factor as compared to the other two treatments. The observed condition might have been caused by the low density of fish in the cage thus better feeding conditions, good appetite, reduced competition for space and reduced stress. Similar findings were reported by Mokoro, (2008).

In this study, FCR was density dependent and higher stocking density resulted to significantly higher FCR compared to the low stocking density. High FCR is an indicator of low food efficiency and has been attributed to the decreasing efficiency in searching for food, poor water quality (Abou *et al*., 2007) and impaired visual location of food (Silva *et al.,* 2000). This finding confirms observations made by Gibtan *et al*., 2008; Opiyo, 2010. However, some authors (Yi *et al.,* 2005; Osofero *et al.,* 2009) have reported no significant difference on FCR. In this study, fish were fed to satiation since the amount of feed given to the fish was based on the rearing density. Therefore, the higher FCR at 100 fish m^{-3} could be attributed to lower growth rates (Yi *et al.,* 1996), poor feeding and poor appetite. Another probable reason is the fact that overcrowding resulted to stress thus food consumed was used to curb stress instead of being converted to somatic growth. FCR in this study was lower than that reported by Gibtan *et al*., 2008 of up to 7.22 in a study to evaluate the effect of stocking density on the growth performance and yield of Nile tilapia in a cage culture system in Lake Kuriftu, Ethiopia.

Fish Survival

The highest stocking density had the lowest percentage survival indicating that survival rate is density dependant which confirms observations made by Huang and Chiu, (1997). This agrees with findings of other fish as illustrated by Aksungur *et al.,* (2007) who reported that stocking density had a significant effect on survival rates of turbot. However this contradicts findings by other authors (Yi *et al*., 2005; Abou *et al*., 2007; Gibtan *et al.,* 2008; Osofero *et al.,* 2009) who noted that survival of *O. niloticus* was not density dependent. The reduced survival rate at high stocking density might have been caused by the crowding of fish stocked at 100 fish m^{-3} which caused stress and voluntary appetite suppression. This might have had a negative effect on the immune system thus the high mortalities. Another probable explanation for the high mortalities in the highly stocked cages is the intense antagonistic behavioural interaction (Chakraborty *et al.,* 2010) which might have increased stress in the fish.

Mortalities were first experienced on the fifth day which coincided with the introduction of supplemental feeds. Increased metabolic rate due to feeding might have been the cause of the mortalities. Mortalities remained high for the first two weeks. This is because fish were not yet acclimatised to the new environment. Another cause could be handling during stocking. Survival of fish has a significant role to play in increasing the revenue and thus profitability of a culture unit and according to Ofori *et al.,* (2009), extreme care must be taken in transportation, holding and handling of fish to avoid heavy mortalities. Mortalities were also experienced after every sampling occasion. This could have resulted from handling during sampling which increased stress in fish and led to mortalities. Percentage survival in all treatments was above 80% and higher than that reported by Ofori *et al.,* (2009) of 30% but lower than that reported by Osofero *et al.,* (2009) of 98.5 – 99.5%.

Yield

In this study, highest yields were recorded in the high stocking density. This indicates that yield is density dependant. Similar findings were reported by Gibtan *et al.,* (2008). Yield was high in the high stocking density due to the high number of fish in the cage as compared to the other stocking densities.

Additional yield was obtained from the pond which added to the revenue accrued from the cages. Fish stocked in the pond showed differentiation in sizes during harvest. This might have been caused by the fact that some fish are fast growers. Another probable reason could have been recruitment. Fish in the pond showed high recruitment since the fish stocked in the pond were mixed sex. Production of fish from the two systems i.e. pond and the cages, can help the farmers to stay in production throughout the year. This can be achieved by stocking the fast growers from the pond in the cage once those in cages attain market size and since there is recruitment in the pond, continued cycle is guaranteed. From this culture system, farmers can diversify in their final products such that they can sell both table size fish and fingerlings to other farmers at the same time.

Water Quality

Measured water quality parameters i.e. D.O., pH, temperature, total phosphorus, SRP, total nitrogen and NO_2-N were within the optimal limits for the growth of *O. niloticus* as recommended by Popma and Masser, (1999). Therefore, the growth rates recorded were not affected by the water quality.

Economic Benefits

The highest returns were recorded in the low stocking density i.e. 50 fish m^{-3} in spite of the low yield and revenue accrued from this treatment. This might have been as a result of the low amount of feeds and fingerlings used in this stocking density as compared to the other two treatments. Returns decreased with increase in stocking density indicating a negative relationship. Breakeven price and breakeven yield increased with increase in stocking density. This could have resulted from the increased variable costs as the stocking density increased. This clearly shows the efficiency of the low stocking density.

REFERENCES

Abou, Y., E. D. Fiogbe and J. Micha. (2007). Effects of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus* L., fed *Azolla* diet, in earthen ponds. *Aquaculture Research* **38**: 595 – 604.

Aksungur, N., M. Aksungur, B. Akbulut and İ. Kutlu. (2007). Effects of Stocking Density on Growth Performance, Survival and Food Conversion Ratio of Turbot (*Psetta maxima*) in the Net Cages on the South-eastern Coast of the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, **7**: 147-152

Ayoade, A. A. and A. O. O. Ikulala. (2007). Length-Weight relationship, Condition factor and stomach content of *Hemichromis bimaculatus, Sarotherodan melanotheron and Chromidotilapia guentheri* (Perciformes: Cichlidae) in Eleiyele Lake, Southwestern Nigeria. *International Journal of Tropical Biology*, **55** (3-4): 969-977.

Baker, R. F. and G. B. Ayles. (1990). The effect of varying density and loading level on the growth of Arctic charr (*Salvelinus aspinus* and rainbow trout (*Oncorhynchus mykiss*). *World aquaculture* **21**: 58 - 62

Boyd, C. E. and C. S. Tucker. (1992). Water quality and pond soils analysis for aquaculture. Alabama Agric. Exp. Station, Auburn University, Alabama, USA.

Chakraborty, S. B., D. Mazumdar and S. Banerjee. (2010). Determination of ideal stocking density for cage culture of monosex Nile tilapia (*Oreochromis niloticus)* in India. *Proc. Zoological Society* **63** (1): 53–59

Coche, A. G. (1979). A review of cage fish culture and its application in Africa. *In:* Pillay, T. V. R. and W. A. Dill (Eds.), *Advances in Aquaculture*. Fishing News Book, Farnham, pp. 428-441.

Diana, J. S. C. K. Lin, and Y.Yi, (1996). Timing of supplemental feeding for tilapia production. Journal of the World Aqua. Soc. 27(4) 410-419.

Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M. and Gadd, D (2002). The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology* **61:** 493–531.

FAO (2010). *The state of the world fisheries and aquaculture 2010.* FAO Fisheries and Aquaculture Department, Rome, Italy.

FAO (2008). *The state of the world fisheries and aquaculture 2008.* FAO Fisheries and Aquaculture Department, Rome, Italy.

FAO (2006). *The state of the world fisheries and aquaculture 2006.* FAO Fisheries and Aquaculture Department. Rome, Italy.

Fitzsimmons, K. and P. Gonzalez (2006). Future expansion of global supplies and markets for tilapia products-2006 *In*: Sanchez, W. M. C. and K. Fitzsimmons (Eds.). *Tilapia, sustainable aquaculture from the new millennium*, Proceedings 7th International Symposium on Tilapia in Aquaculture

Gibtan, A., A. Getahun and S. Mengistou (2008). Effects of stocking density on the growth performance and yield of Nile Tilapia [*Oreochromis niloticus* (L., 1758)] in a cage culture system in Lake Kuriftu, Ethiopia. *Aquaculture Research* **39**(13)**:** 1450-1460.

Huang, W. B. and Chiu, T. S. (1997). Effect of stocking density on survival, growth, size variation and production of tilapia fry. *Aquaculture Research* **28**: 165 – 173.

Lin, C. K. and J. S. Diana. (1995). Co-culture of Catfish (*Clarias macrocephalus x C. gariepinus*) and Tilapia (*Oreochromis niloticus*) in Ponds. *Aquatic Living Resources* **8**:449-454.

Liti, D. M., B. Fulanda, J. M. Munguti, M. Straif, H. Waidbacher, and G. Winkler. (2005). Effects of open-pond density and caged biomass of Nile Tilapia (*Oreochromis niloticus* L.) on growth, feed utilization, economic returns and water quality in fertilized ponds. *Aquaculture research* **36:** 1535 – 1543.

McGinty, A. S. and J. E. Rakocy (1989). *Cage Culture of Tilapia.* South Regional Aquaculture Center (SRAC), Publication No. 281.

Mokoro, A. K. (2008). Effects of stocking density on growth and survival of Nile Tilapia (*Oreochromis niloticus*, Linn. 1858) in cages. M. Phil thesis, Moi University, Kenya.

Ndimele, P. E., C. A. Kumolu- Johnson, N. F. Aladetohun, and O. A. Ayorinde, (2010). Length-weight relationship, condition factor and dietary composition of *Sarotherodon melanotheron*, Rüppell, 1852 (Pisces: cichlidae) in Ologe Lagoon, Lagos, Nigeria. *Agriculture and Biology Journal of North America*, **1**(4): 584-590

Opiyo, M. A. (2008). Influence of rearing density and background colour on the growth performance and survival of Nile tilapia (*Oreochromis niloticus* - Linn, 1758) fry M. Phil thesis, Moi University, Kenya.

Osofero, S. A., S. O. Otubusin and J. A. Daramola. (2009). Effect of stocking density on tilapia (*Oreochromis niloticus,* Linnaeus 1757) growth and survival in bamboo-net cages trial. *African Journal of Biotechnology,* **8**(7): 1322-1325

Petit, G., Beauchaud, M. and Buisson, B. (2001). Density effects on food intake and growth of Largemouth bass (*Micropterus salmoides*). *Aquaculture Research* **32**: 495 – 497.

Popma, T. and M. Masser. (1999), *Tilapia: Life History and Biology*. South Regional Aquaculture Center (SRAC), Publication No. 283.

Quagrainie, K. K., C. C. Ngugi, and S. Amisah. (2009). Analysis of the use of Credit facilities by small – scale fish farmers in Kenya. *Aquaculture International,* **18**:393-402

Shrestha, M. K. (2002). Mixed sex Nile tilapia culture model for subtorial Nepal. *In*: *Book of Abstracts-World Aquaculture 2002*, Beijing, China. pp. 699-699.

Silva, P. C., V. L. Souza, D. M. Padua, P. C. Dalacorte and D. C. Goncalves. (2000). Effect of stocking density on growth and fillet composition of tetra hybrid red tilapia, Israeli strain. *In:* Fitzsimmons K. and Filho C. (Eds.), *Tilapia Aquaculture in the 21st century*: Proceedings from 5th International Symposium on Tilapia Aquaculture, pp 341-345. The World Aquaculture Society, FL, USA.

Yi, Y., N. T. Phuong, J. S. Diana and C. K. Lin. (2005). Integrated cage-cum-pond culture systems with high-valued climbing perch (*Anabas testudineus*) in cages suspended in Nile tilapia (*Oreochromis niloticus*) ponds. *In*: J. Burright, C. Flemming, and H. Egna (Eds.), Twenty-Second Annual Technical Report, Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp.115-131.

Yi, Y. and C. K. Lin. (2001). Effects of biomass of caged Nile tilapia (*Oreochromis niloticus*) and aeration on the growth and yields in an integrated cage-cum-pond system. *Aquaculture* **195**, 253-267.

Yi, Y. and C. K. Lin. (2000). Analysis of various input for pond culture of Nile Tilapia (*O. niloticus*): Profitability and potential environmental impacts. *In*: Fitzsimmons, K. and J. C. Filho (Eds.), *Tilapia Aquaculture in the 21st century* Vol. I, Proceeding from the fifth International symposium on Tilapia Aquaculture, Sept. 3-7, 2000, Rio de Janeiro, Brazil. pp. 247-257.

Yi, Y., (1997a). A cage-cum-pond integrated rotation culture system for fattening large Nile tilapia (*Oreochromis niloticus*) in cages and nursing small Nile tilapia in open ponds. Ph.D dissertation, Asian Institute of Technology, Bangkok, Thailand, 169 pp.

Yi, Y., (1997b). A bioenergetics growth model for Nile tilapia (*Oreochromis niloticus*) based on limiting nutrients and fish standing crop in fertilized ponds. Thailand Special Topics Research 1, Thailand. *In*: Burke, D., J. Baker, B. Goetze, D. Clair, and H. Egna (Eds), Fifteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon (pp 140-167).

Yi, Y., C. K. Lin, and J. S. Diana. (1996). Effects of stocking densities on growth of caged adult Nile tilapia (*Oreochromis niloticus*) and on yield of small Nile tilapia in open water in earthen ponds. *Aquaculture*, **146**:205-215.

Expansion of Tilapia and Indigenous Fish Aquaculture in Guyana: Opportunities for Women

Sustainable Feed Technology/Study/09SFT03UA

Pamila Ramotar
Department of Fisheries
Georgetown, Guyana

Kevin Fitzsimmons
University of Arizona
Tucson, Arizona, USA

ABSTRACT

The focus of this investigation was an effort to work with fish farmers in two regions of Guyana to develop sustainable aquaculture systems. In the coastal region we worked with the Trafalgar Union women's cooperative and some individual farmers. Their primary focus was on tilapia and hassar. The hassar is a native species of armored catfish that is considered a delicacy in Guyana and Trinidad and Tobago. Tilapia were introduced to Guyana many years ago for mosquito control but have now become a popular food fish. In recent years, genetically male tilapia have been imported from Great Britain to upgrade the broodstocks available. We collaborated with the Guyana Department of Agriculture on the distribution and maintenance of these broodstocks to a regional hatchery.

In the interior region, we have been working with the Bina Hill Association which is composed primarily of women representatives for several of the surrounding villages. Our primary focus was on working with these women and the rest of the community to develop and demonstrate an integrated aquaculture-agriculture system that would be stocked with native fish species. With no electrical grid available, we determined it would be better to run from solar panels and batteries, rather than the very expensive diesel which must be trucked in. The first solar panel and battery array was purchased and delivered to the demonstration. The fish are scheduled to be delivered in late September 2011, assuming the rainy season has ended and the road is passable.

Over all we conducted three workshops for the women of Trafalgar Union and one farm visit. We held three workshops for the Bina Hill community in Annai and have prepared the plans for the demonstration and provided some of the materials. We have also provided some of the aquaculture supplies for both projects.

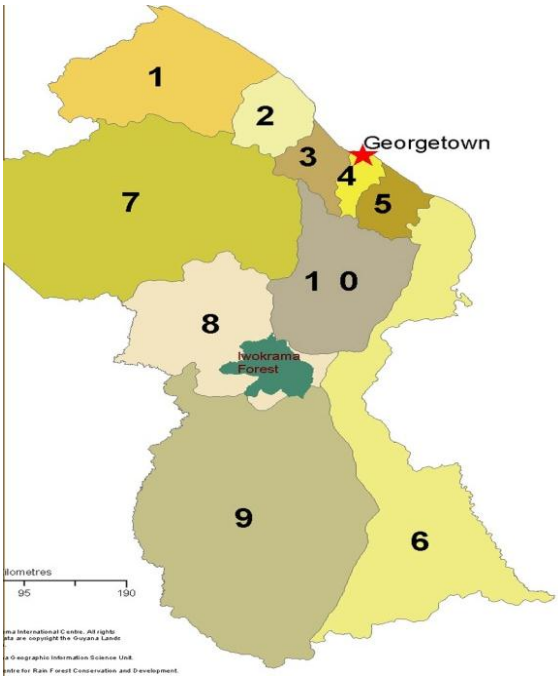
OBJECTIVES

a. To support development of small scale aquaculture in poor rural areas, with a focus on women's groups in Trafalgar Union and Annai
b. To support development of a regional hatchery program utilizing YY Genetically Male donated by DFID
c. To identify and determine indigenous fishes with aquaculture potential
d. Finalize development of a standard aquaculture feed containing locally available ingredients.

INTRODUCTION

Guyana, in South America, has some of the best protected rainforests in the world as well as enormous quantities of pristine freshwaters, little industry, a relatively small population and a long coastline. There is tremendous potential for small and large scale aquaculture in freshwaters, brackish water and in marine systems. The northern watersheds drain to the Caribbean while its southern watersheds are part of the Amazon Basin. Our AquaFish CRSP work in Guyana is split between the two areas. The northern watersheds drain to the coastal areas to the east and west of the capital Georgetown. The road to the west does not extend very far into Region 3, but the east road extends all the way to the border with Surinam. In this eastern area, Regions 4 and 5, we have worked with a number of individual farmers rearing tilapia and pacu and with one women's cooperative rearing tilapia and hassar, a local armored catfish. The farms are all arrayed along the coastal highway and use their fish primarily for direct consumption and local sales, as well as some sales to the major population center of Georgetown.

ADMINISTRATIVE REGIONS OF GUYANA



Region 1	Barima-Waini
Region 2	Pomeroon-Supenaam
Region 3	Essequibo Islands-West Demerara
Region 4	Demerara-Mahaica
Region 5	Mahaica-Berbice
Region 6	East Berbice Corentyne
Region 7	Cuyuni-Mazaruni
Region 8	Potaro-Siparuni
Region 9	Upper Takutu-Upper Essequibo
Region 10	Upper Demerara-Berbice

RESULTS

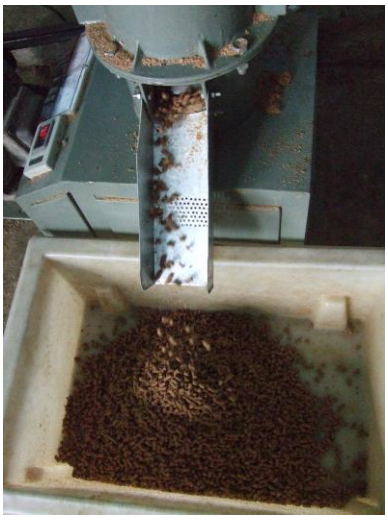
In Regions 4 and 5 we have worked with most of the individual farmers including those growing tilapia, pacu and shrimp, a feed mill producing fish feed and one tilapia hatchery. The tilapia farmers are using both Mozambique tilapia imported years ago and improved selections of Nile tilapia donated by Swansea University in Wales with support from the British DFID. The YY genetically male tilapia were first brought to the Mon Repos Station managed by Pamila Ramotar with the Fisheries Office. Later some of the broodstock were transferred to Mr. Chico Persaud, operator of the Maharaja Mill. Chico was the manufacturer of the local aquaculture diets and our collaborator on feed formulations and trials. We helped to design a tilapia hatchery adjacent to his feed mill. Unfortunately after all of our work with Mr. Persaud, he emigrated to Canada in late 2010. He has left the Mill and hatchery in hands of a brother who has no background in the business and has assigned a caretaker to the operation. We have spent some time to educate the caretaker, but so far with slow progress.

Tilapia hatchery at Maharaja Mill

Due to the departure of Mr. Persaud, our feed development aspect, and trials, was hindered. The eventual decision was to have Ms. Ramotar continue the feeding trials as part of her Master's project here at the University of Arizona. The trials are underway at the time of this writing.

Lacking access to the Maharaja Mill, we did develop a small project with Mr. Benni Sankar to develop simple on farm diet with local ingredients. Mr Sankar purchased a small hammer mill grinder and a basic pellet mill. Both pieces of equipment were manufactured in China and came without any instructions. We spent several days working with Mr. Sankar at his farm in Region 3. His basic ingredients were to be fish meal derived from the carcasses of filleted marine catfish, rice bran and broken rice from the rice farm operation and vegetable oil. We spent considerable time getting the equipment operating, training staff, and formulating a mix that would go through the equipment without plugging dies and screws. We eventually came up with a workable formulation. But the stability of the pellet in water was very short, less than one minute.

Simple hammer mill for grinding fish carcasses



Pellet mill

We have more expansive hopes for the additional farmers in Region 4 and 5 and have started test shipments of tilapia to Florida. The first shipment was stalled and ruined by airline employees who thought that we were missing a permit. The second shipment was successful with high quality fish shipped to and sold by Sammy's Seafood, a seafood company in St. Petersburg, FL . We have also assisted the two shrimp farmers who use extensive methods of non-fed, tidal impoundments. On our last visit we collected shrimp samples for return to Arizona for species identification and gross health exam. We have suggested that they consider tilapia in cages in their ponds to increase revenues, increase growth and survival of the shrimp and diversify their sales (in time and product).

Pamila Ramotar with farmed pacu

Preserving shrimp samples

The Trafalgar Union Women's Cooperative is one of the largest farms in Region 5. This cooperative of 16 women have pooled their resources and been provided with a low cost lease on 12.5 hectares of federal land. The farm now includes 10 ponds and four more under construction. We have conducted three workshops for the women. One at the national aquaculture center at Mon Repos, one at the Maharaja Feed Mill and a third at the Trafalgar Union Farm. The first workshop, 15 August 2008, included basics of aquaculture and tilapia biology. The group met early in the morning and we started with introductions and welcoming comments. We spent the rest of the morning covering aquaculture production systems and marketing. We had a lunch provided for everyone and then started an afternoon session focused on tilapia and the two strains, YY Nile tilapia and red tilapia that are currently being farmed. Later in the afternoon we toured the Mon Repos facility and then reconvened at the Maharaja Oil Mill. We discussed some basics of feed manufacturing and feed handling. The second workshop, 18 June 2009, was held at the Mon Repos station and also moved later to the Maharaja Mill and covered basic fish nutrition, feed formulations, feed manufacturing, and on farm feed handling and distribution. The third workshop was held at the Trafalgar Union farm on 20 June 2011. This workshop was conducted on the farm moving from site to site. We started at the pumping station for introductions and to review water supplies and biosecurity. We then all moved to the ponds to discuss feeding practices, predator control, weed and algae control, taste and odor issues and harvest practices. Finally we moved back to the office and working area to discuss harvest and quality control aspects. We also discussed the sales and marketing aspects and plans for international distribution (to Florida and Trinidad).

Office and feed storage at The Trafalgar Union Model Farm. Coop leader Ms. Shenella Lewis and several of the sons and nephews who have been hired to work at the farm.

Our second area of interest is in the Rupununi Basin in the southern portion of Guyana. The Rupununi is a part of the Amazon watershed that seasonally floods large areas of savanna. The indigenous populations of the Rupununi are commonly referred to as AmerIndians. These native people have a very different culture and lifestyle compared to the coastal populations, which are composed of the descendants of black slaves and East Indian indentured workers. There is only one unpaved road across the Rupununi Basin and no electrical or phone systems. The people are essentially subsistence farmers utilizing solar panels or diesel generators for household electrical power. As there are no phone, radio or television signals, Internet connections through satellite dish is the only communication with the outside, besides irregular mail service when the dirt road is passable.

Our focus in the southern watersheds was to develop, describe and demonstrate a simple integrated farming system utilizing native fishes and vegetable crops grown in the area. We organized our workshops and visits with the Fisheries Office staff in Georgetown before flying by small plane to the airstrip at Annai. We held our first workshops in August 2010 to describe the system to the community members and to gather their input and suggestions as to how to improve the concept. We also visited several ponds that had previously been built in the area. None of the ponds could be drained. None were fenced to keep out caiman or other fish eaters. Several of the ponds were more than three meters deep. The attempts at stocking had failed. And any attempts to harvest by net were sure to fail.

Therefore we recommended a simple small pond system coupled with production of local vegetables. We also spent considerable time discussing the native fishes that could be farmed and how best to feed them in a farm setting. The species of greatest interest were: pacu (*Colossoma)*, cascadura (*Hassar*), and arapaima (*Arapaima gigas*). All three are heavily overfished in the area and are considered delicacies of high monetary value. The pacu and especially arapaima are considered excellent sport fish that support valuable tourism. After the workshop we conducted a literature review to examine other species and to gather culture information on the pacu, hassar and arapaima.

Poorly constructed pond

Community pond used for fishing

A second workshop was led by our host country PI, Pamila Ramotar on 21 September, 2010 and a third on 12 November 2010. She presented additional information of culture of pacu and arapaima with handouts and contacts to Brazilian farms and hatcheries.

With the lack of reliable electricity we incorporated a solar panel for the pump to be used during irrigation periods during our fourth workshop on the 15th and 16th of June 2011. For the demonstration pond and garden, a panel was purchased in Georgetown along with a battery, a controller and a direct current pump. We took the materials as our checked baggage and flew to Annai with change of clothes in hand.

After the prior workshop and discussions with the local association of farmers, we determined to put the demonstration farm at the Rock View Lodge. The Lodge is situated between the airstrip and the dirt highway crossing the Rupununi. The regional primary and secondary schools are on either side of the Lodge and virtually all the school children cross the property twice a day going or returning from school. With the open and sharing nature of the society, it is normal for everyone to stop and see anything that is new when "passing by". Everyone agreed this would be the best location. The proprietor, Colin Edwards, has started the pond construction and planted the garden. He has the solar panel and battery apparatus ready to install when the fish are stocked. The current plan is to stock with pacu fingerlings. The pacu are native to the area and fingerlings are available from a hatchery across the border in Brazil. Delivery is only possible in the dry season, but plans have been developed for stocking in November 2011.

During the fourth workshop, we also spent considerable time discussing within the community the pro's and con's of rearing tilapia versus the native fishes. While most of us would prefer to work with native fishes, one member of the community felt that tilapia would be a superior fish and insisted on his right to import and rear the fish. A considerable amount of time was spent on this issue. We think we finally convinced the farmer that natives were a preferred crop for now and that the introduction of tilapia into the region had too many potential environmental risks.

Workshop in Annai, at the Bina Hill Community Center, August 2010.

CONCLUSIONS

The project has certainly succeeded in providing the training and support to assist two groups and several individuals to advance their aquaculture efforts and improve sales of farmed fish domestically and now on an international level. In addition to training we have provided some materials and supplies for their operations. We have worked closely with the Guyana Investment and Trade Service, a contractor working on US-AID grant to support development in Guyana. We have also coordinated with the Farmer to Farmer program who provided support for one of our graduate students to contribute with a volunteer mission to Guyana.

We expect to see continued growth of the aquaculture sector in Guyana with tilapia, hassar and shrimp along the coast and with pacu and arapaima in the interior. Our contributions to training, technical support, supplies and international marketing are showing results. We look forward to additional expansion in coming years. A significant weakness in the project has been the half-hearted support of the Department of Fisheries within the Ministry of Agriculture. At times, Fisheries staff assigned to work with us were not allowed to travel to international conferences, even with our full financial support, or even to accompany in the field within Guyana, again with our financial support. Some of the monies transferred to support specific activities within Guyana, have still not been spent. These funds are still in a suspense account that can be spent as soon as approved by the Minister, but repeated assurances that the approval was pending and the funds would be spent as promised have come and gone.

Feeding and Feed Formulation Strategies to Reduce Production Costs of Tilapia Culture

Sustainable Feed Technology/Experiment/09SFT04NC

Remedios Bolivar
Central Luzon State University
City of Muñoz, Philippines

Peter Ferket, Charles Stark and Russell Borski
North Carolina State University
Raleigh, North Carolina, USA

ABSTRACT

Feed constitutes 60-80% of total production costs of tilapia (*Oreochromis spp.*). Reductions in quantity of feed used for fish growout and in the cost of formulated feeds are two approaches to containing feed costs. We evaluated combined feed reduction strategies, and less costly fishmeal-free and low crude protein diets as well as feed manufacturing options for improving production efficiency of Nile tilapia (O. niloticus). In a 120-day pond trial, fish subjected to a combined feed reduction strategy that incorporates a sequential series of a 60-day, 67% feeding rate with a 60-day 50% daily or 100% alternate day feeding rate had similar growth rates as fish fed daily at the 67% level throughout the trial. However, they exhibited lower survivorship, and hence the final extrapolated yield at harvest of animals on the combined feed reduction protocol was significantly lower. It appear sthat this protocol of incorporating a combination of feed reduction stategies is less effective than any of the strategies used alone, which were previously shown to reduce feed costs by as much as 56% in the growout of marketable tilapia. In a second pond trial we show that elimination of fishmeal from a standard 31% CP diet can produce a significant costs savings in feeds with no appreciable effect on growth performance and yield of tilapia raised in ponds. This confirms our earlier report whereby fishmeal-free diet provide an additional 8% cost savings in growout of tilapia over and above the > 50% savings seen with an alternate day feeding protocol. A reduction of dietary CP to 26%, so long as the amino acid composition remains similar to that of the higher 31% CP feed, can produce additional cost savings in feed of up to a 5.69% if fishmeal is included in the diet or up to 11.69% if fishmeal is not included in the lower protein diets. Hence, this work demonstrates that both elimination of fishmeal and reductions in the amount of crude protein or both significantly reduces the costs of feeds for growout of tilapia with little overall impact on growth parameters, survival or yield of fish. We also demonstrate that the production of a less costly pelleted fishmeal-free diet over an extruded form is further effective in reducing feed costs with negligible impact on performance. This was particularly true of the higher 31% CP diet. The ability to produce pelleted fishmeal-free aquafeeds that are of comparable performance as extruded feeds can not only promote an additional cost savings in feed of 3-7%, but would allow manufacturers who lack costly extruders to expand to production of aquafeeds. Collectively these series of studies and our previous work demonstrates that over and above any effects of reducing the amount of feed applied to the culture of tilapia, that up to 18% savings of feed costs can be achieved with fishmeal-free, lower crude protein and pelleted diets relative to the standard higher protein extruded feed containing 6% fishmeal.

INTRODUCTION

Feed is the most costly component of fish farming, accounting for as much as 80% of total production costs for small-scale, rural farmers in the Philippines that grow tilapia (ADB 2005; El-Sayed 2006). Approximately 35% of all feed manufactured or sold in the Philippines is for aquaculture, largely for

tilapia. Provided production efficiency is not significantly impaired, any reductions of feed costs per kg of fish marketed will increase income at several levels of Philippine society, including local suppliers of feedstuffs, feed manufacturers, allied industries, and especially tilapia farmers. Total feed costs for tilapia can be reduced through three approaches: 1) decrease in the amount of feed used for grow-out of marketable fish; 2) decrease feed formulation costs by reducing crude protein levels, amino acid supplementation and replacing expensive fish meal with lower cost protein sources; and 3) reduce feed manufacturing costs by pellet processing in place of the more expensive current practice of extrusion processing. The objectives of this investigation will address these three approaches to reducing feed costs for Filipino tilapia farmers.

Our previous studies show that 1) delaying the onset of supplemental feeding to either 45-days or 75-days in fertilized ponds reduces the amount of feed consumed without any negative impact on the production of marketable tilapia, 2) feeding daily at 67% subsatiation produces growth rates identical to that of fish fed daily at full feeding levels and significantly improves production efficiency of tilapia growout, and 3) feeding daily at 50% satiation or on alternate days at the prescribed satiation level saved approximately half of feed cost without a significant reduction in growth, survival, or market yield of Nile tilapia in growout ponds (Brown et al. 2000, Bolivar et al. 2003, Bolivar et al. 2006; Bolivar *et al.*, 2010). Nevertheless, fish were smaller on average at harvest with 50% daily satiation or alternate day satiation feeding relative to animals fed daily at the prescribed full level. Therefore, we tested a combined feeding strategy to evaluate whether a 67% subsatiation feeding combined with alternate day or 50% reduced feeding could reduce costs with no impact on growth or total biomass produced (see footnote at end of text).

The formulation cost of commercial diets has risen sharply with about 40% of aquaculture feed costs attributable to fishmeal that constitutes as much as 20% of feed formulation. Much of the fishmeal used in the Philippines is imported and costs are expected to continue to rise as global supplies decline and demand increases. Unlike carnivorous species, tilapia are omnivorous fishes that do not require fish in their diet, and they are an ideal group of species to recycle food by-products into high quality food protein for humans (Brown 1983). Moreover, tilapia can digest a relatively high carbohydrate diet, and effectively utilize lower-cost feed ingredients readily available in the Philippines (e.g. rice bran, copra meal, and cassava) to completely replace or significantly reduce fishmeal use (Jackson et al. 1982; NRC 1993). Indeed, various animal and plant proteins have been shown to be either partially or completely replace fishmeal in tilapia diets (Lim and Webster 2006; El-Sayed 2006). For example, we reported tilapia fed diets with up to 33% sweet potato and lactic acid-stabilized poultry carcasses did not adversely affect tilapia growth performance or consumer panel sensory indices (Middleton et al. 2000). Additionally, our previous AquaFish CRSP research demonstrates that fishmeal can be replaced with poultry by-p*roduct meal, fermented poultry protein, or yeast extract protein without adversely affecting growth performance of tilapia fed pelleted feed (Ayoola 2010; Bolivar et al. 2010). Likewise, we have demonstrated that dietary replacement of a standard diet containing 6% fishmeal with porkmeal does not adversely effect growth of tilapia fed on alternative days in ponds, but produces a 8% cost savings in feeds (Borski et al. 2011). As observed with other monogastric omnivorous animal species, feeding lower protein diets supplemented with amino acids could significantly reduce excess nitrogen emission into pond water and increase profit margins by lowering feed costs for tilapia farmers (Ferket et al. 2003). These studies will evaluate the production performance feasibility of tilapia fed extruded high crude protein diets versus a lower crude protein diets supplemented with amino acids, and that contain either 0% or 6% fishmeal.

Most commercial aquaculture feed, including those for tilapia, is manufactured and fed as floating or slow-sinking extruded pellets because it is easier for fish farmers to detect over feeding and it has good water stability and durability. However, extrusion processing costs are higher than pellet processing. Likewise transport costs per ton are higher for extruded feeds because of their lower bulk density. Our previous work

at NCSU have shown that tank-reared tilapia are able to consume pellet-processed feed without adversely affecting growth performance efficiency (Ayoola 2010; Bolivar et al. 2010). The economic and production feasibility of feeding pellet processed feed must be confirmed with pond-reared tilapia in the Philippines. However, more research must be done to improve the water stability and durability of pelleted feed for pond systems.

Pond-reared tilapia feed must have sufficiently high water stability that allows feed to be consumed by the fish and prevent nutrients from dispersing or leaching in the water, leading to poor feed conversion and performance (Leonard et al. 2002). Diet formulation, ingredient particle size, conditioning, die specs, and cooling are the major factors that influence pellet quality (Axe 2002). Proteinaceous and starch materials have the greatest influence on producing a water-stable feed, as well as the affecting the formula costs to meet nutrient specifications (Rokey and Huber 2005). However, most by-product protein meals commonly used in tilapia feed are heat-denatured proteins, which have reduced pellet-binding characteristics. In contrast, dietary inclusion of nutritive protein binders from yeast, animal, and plant sources or pelleting aids, could significantly enhance the durability of pelleted feed for tilapia. In the proposed investigation, we will evaluate the effect of dietary inclusion of various nutritive protein pellet binders and pelleting aids on the pellet durability and water stability of pelleted feed. This information will then be used to formulate and manufacture pelleted tilapia feed for pond culture systems and compare it to conventional extrusion-processed feed in terms of production efficiency and profitability.

OBJECTIVES

1. Evaluate effects of combined feed reduction strategies on the growout performance of Nile tilapia in fertilized earthen ponds.
2. Evaluate pond growout performance of Nile tilapia fed high and low crude protein diets with and without fishmeal.
3. Evaluate dietary inclusion of nutritive protein binders and pellet processing aids on pellet durability and water stability of tilapia feeds.
4. Compare the effect on tilapia feed manufactured by extrusion and pellet processing on pond grow-out performance of Nile tilapia.

MATERIALS AND METHODS

Study 1 - Evaluation of 67% subsatiation daily feeding combined with 100% alternate day or 50% daily feeding versus continuous 67% daily feeding on growout of Nile tilapia in earthen ponds

Previous studies show that feeding at 67% of normal rates produces identical growth rates to animals fed at the typical 100% level, but at a signficant cost savings infeeds. This study evaluated if the 67% subsatiation feeding when combined with previous feed reduction strategies of alternate day feeding and 50% subsatiation might produce an additional cost savings with little impact on growth performance. This study evaluates three feeding treatments on the growth performance of Nile tilapia as follows: Treatment I – 67% daily feeding until harvest; Treatment II - 67% daily feeding for 60 days followed by 50% daily feeding until harvest; and Treatment III - 67% daily feeding for 60 days, followed by 100% alternate day feeding until harvest.

Sex-reversed Nile tilapia (*Orechromis niloticus*) fingerlings (#20; 0.35- 0.37 g) were stocked at 4 fish m^{-2} in nine 500-m^2 earthen ponds at the Freshwater Aquaculture Center (FAC), Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija, Philippines with three replicates per group. The grow-out phase of this study were done in eight 500 m^2 earthen ponds. Size 20 (weight range =) fingerlings of the GIFT strain were stocked in each pond. Fish were fed with pre-starter feeds containing 34% crude protein (CP) for the first month, starter feeds with 34% CP over the second month, and then grower feeds containing 31% CP until harvest. Treatment feed rations were adjusted based on the full

ration levels prescribed that ranged from 20% down to 2% average body weight over the course of the study. Feeding adjustment was done every two weeks when average body weights of fish were ascertained. The amount of feeds used per treatment was recorded daily. Fish sampling was done every two weeks by bulk weighing of 100 fish in each ponds. Individual weight and length of 100 fish were measured at the onset and termination of the study 120 days post stocking.

Water temperature and dissolved oxygen were measured weekly at 0900 and 1300 hr using dissolved oxygen meter (YSI model 55). Hydrogen ion concentration (pH) and Secchi disc visibility depth (SDVD) reading was also measured weekly. Determinations of the other water quality parameters (total ammonia nitrogen and nitrite-nitrogen level) were measured using freshwater test kit (Lamotte Model AQ2). Ponds were fertilized with ammonium phosphate (16-20-0) and urea (46-0-0.) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water. Weekly fertilization of the experimental ponds was adjusted based on SDVD.

After 120 days of culture, stocks were harvested for bulk weight of all the stocks, survival rate, and the extrapolated gross fish yield (total weight of fish at harvest (kg) / area of the pond (m^2)). Other variables, including total feed consumption, feed conversion ratio (FCR; feed consumed/body weight), specific growth rate (SGR; % daily body weight and length gain; $[(\ln W_f - \ln W_i)/(T_f - T_i) \times 100]$) were calculated. Differences among means were statistically analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Study 2 – Evaluation of pond growout performance of Nile tilapia fed high and low crude protein diets with and without fishmeal.

This study was composed of 4 treatment groups:

1. Feeding of high CP (31%) extruded feed with 6% dietary inclusion of fishmeal – a standard diet used in industry;
2. Feeding of low CP (26%) extruded feed with 6% dietary inclusion of fishmeal supplemented with amino acids
3. Feeding of high CP (31%) extruded feed without (0%) dietary inclusion of fishmeal
4. Feeding of low CP (26%) extruded feed without (0%) dietary inclusion of fishmeal supplemented with amino acids

Diets were least-cost formulated to be similar to those from our previous work that demonstrated 31% CP fishmeal-free feed was as effective on the performance of tilapia in ponds as a standard diet containing 6% fishmeal (Borski et al. 2011)(Table 3). Diets were composed of locally available ingredients in the Philippines that included hydroyzed animal protein with fishmeal or porkmeal. Locally produced plant protein sources included copra cake, cassava, and rice bran. Soybean meal, upon which world protein market prices are based, was used as the basal plant protein source because of consistency of availability and nutritional quality. All grower diets were formulated to meet the nutrient requirement of tilapia (NRC 1993; Li et al. 2006) using least-cost linear programming. The low protein diets were supplemented with commercially available amino acids such that they have a similar amino acid profile as the high crude protein diet. Diets were formulated by Ms. Ning Pascaul of our industry cooperator, Santeh Feed Corporation (Tateh Aquafeeds, Santeh Feed Corp. Quezon City, Philippines).

Sex-reversed Nile tilapia fingerlings (#22, 0.162 g body weight) of GIFT strain were stocked at 4 fish m^{-2} in twelve 500 sq. m. earthen ponds at FAC-CLSU, Science City of Muñoz, Nueva Ecija, Philippines, with 3 replicate ponds per treatment group. Fish in all treatments were fed first with pre-starter feeds containing 36% crude protein for the first month and then with starter feeds containing 34% crude protein for the second month. Fish were subsequently fed grower treatment diets until harvest at 120 days poststocking. Fish were fed according to a standard schedule of 20% down to 2% body weight from the

beginning to the end of the pond study. Feeding adjustment was done every two weeks following subsampling for average body weight of stocks.

Fish sampling was done every two weeks by getting the bulk weight of 50 fish samples. Individual weight and length of 100 fish was measured on the initial and final sampling. Water quality parameters were also measured as described above. Water temperature (^{o}C), dissolved oxygen concentration (mg/l), hydrogen-ion concentration, total alkalinity (mg/l), total ammonia nitrogen (mg/l), carbon dioxide concentration (mg/l) and total phosphorus (mg/l) were monitored weekly. Total hardness (mg/l) was monitored monthly. Ponds were fertilized weekly with ammonium phosphate (16-20-0) and urea (46-0-0.) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water and adjusted based on SDVD.

Growth and production parameters were measured as described above. Differences among means were statistically analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan 1955).

Study 3 – Evaluate dietary inclusion of nutritive protein binders and pellet processing aids on pellet durability and water stability of tilapia feeds.

The purpose of this study was to evaluate the efficacy of different types of pellet binder feed additives on the durability and water stability of pelleted tilapia feed. A 26% crude protein tilapia basal diet (containing ~34% corn, 25% soybean meal, 22% rice bran, 10% poultry by-product meal, 5% distillers dried grains with solubles, 0.5% poultry fat, and amino acids, vitamins and minerals making up the balance, Table 6) was ground through a #4 hammer mill screen to obtain a 400 micron geometrical mean particle size.

Experiment 1

In experiment 1, the basal diet was split into 4 kg batches. Three commercial pellet binders and one "no binder" control were prepared in triplicate and arranged in three randomized blocks of consecutive processing runs through a small experimental pellet mill with 40 psi steam at ~80C with a 3.7 mm X 18 mm die). Feed additive pellet binders and inclusion levels tested were 0.2% urea-formaldehyde (UF), , 0.1% bone gelatin (G), and 2% wheat gluten (WG). The binders were added to 4 kg of the basal feed according to the manufacturer's recommendations. The feed was pellet with a laboratory pellet mill. Feed was conditioned with 40 psi steam to 80^{o}C and pelleted through a 3.7 mm x 18 mm ring die. Batches were run in immediate sequence with no change in steam or feeder screw rates. The results of this experiment were used to select the pellet binders in Experiment 2.

Experiment 2

The basal tilapia grower formula and three different binders used in experiment 1 were also used here, with the addition of two combined treatments: 0.2% UF + 2% WG and 0.2% UF + 0.1% G. The combination of binders was based on the pellet quality results of Experiment 1. Diets were produced using a commercial-sized pellet mill equipped with a 4 mm X 45 mm die. The feed was conditioned for approximately 30 sec at each target temperature, using a 46 cm x 122 cm conditioner operating at 60 RPM. The pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) was equipped with a 4.4 mm x 35 mm ring die.

Pellet Quality Analysis

Samples in Experiment 1 were tested for pellet compression strength, which was determined with a manually operated compression pellet tester (Amandas Kahl Nachf, 2057 Reinbek. Germany). The hardness of 15 pellets, randomly chosen from each treatment, was measured. Samples in Experiment 1 and 2 were tested for pellet quality with the following testing methods: 1) Pellet durability was determined by duplicate runs on the New Holmen Pellet Tester (NHPT, 68 mbar), whereby 100 g of

whole pellets were tumbled for 30- 90 seconds in a Holmen Pellet Tester, and percentage of remaining whole pellets calculated; 2) KSU pellet durability index (PDI) according to ASAE Method S269.7; 3) modified ASAE method that included the addition of three 19 mm hex-nuts (MPDI); and 4) water stability, which was determined with duplicate 50 g samples of pellets of each diet (PWS). Water stability samples were placed on a 3 mm diameter sieve and slowly immersed in deionized water at 24 ºC for 10 min; the sieve was removed from water, drained for 1 min, oven-dried at 105 ºC for 3 h, cooled in a desiccator, and reweighed. Water stability was calculated as the percentage difference in dry weight.

Study 4: Compare the effect on tilapia feed manufactured by extrusion and pellet processing on pond grow-out performance of Nile tilapia.

In this study we evaluated the effect of using a sinking pellet versus extruded feed on tilapia growout in ponds. Fish stocks were fed with the experimental feeds using a factorial arrangement of 2 manufactured feed forms, floating extruded versus slow sinking pelletd feed with high and low crude protein level. Binders used for the pelleted feed were those shown to maximize pellet durability and stability from Study 3 (0.2% urea-formaldehyde + 0.1% gelatin). All diets were formulated without fishmeal at either low or high crude protein levels similar to Study 2 using least-cost linear programming. Diets were formulated by Ms. Ning Pascaul of our industry cooperator, Santeh Feed Corporation (Tateh Aquafeeds, Santeh Feed Corp. Quezon City, Philippines). The following represent the four different fishmeal-free treatment diets used in the experiment:

I) 31% CP floating extruded feed
II) 31% CP slow-sinking pelleted feed + binders
III) 26% CP floating extruded feed supplemented with amino acids
IV) 26% CP slow-sinking pelleted feed supplemented amino acids + binders

Sex-reversed fingerlings of size #22 (0.170 g/pc) were stocked in twelve 500 m^2 earthen ponds at FAC-CLSU at 4 pcs m^{-2} with 3 replicates per treatment. Fish stocks in all treatments were fed first with fry mash (31% CP) for the first 30 days and starter feeds (30% CP) were given up to 75 days of culture period. Fish were subsequently fed the grower treatment diets aon alternate days for 60 days or until the end of the 135-day growout trial. Alternate day feeding was previously shown to reduce production costs of tilapia without significantly altering final yield as almost 50% less feed could be used to grow fish than that incorporating standard daily feeding practices (Bolivar et al. 2006). Moreover, our previous work showed that replacement of fishmeal with pork meal is as effective in producing tilapia under an alternate-day feed reduction strategy, as those diets containing standard levels of fishmeal (Borski et al. 2011). Feeding rates in the experiment started at 20% and was reduced to 2% based on average body weight of the stocks within the culture period.

Fish sampling was done every two weeks by cast net to obtain average weight of fish stocks determined by bulk weighing 100 fish. Individual weight and length of 100 fish was measured on the initial stocking and final sampling. Water quality parameters were also measured as described above. Water temperature (^oC), dissolved oxygen concentration (mg/l), hydrogen-ion concentration (pH), total alkalinity (mg/l), total ammonia nitrogen (mg/l), carbon dioxide concentration (mg/l) and total phosphorus (mg/l), and SDVD were monitored weekly at 0900 hour. Ponds were fertilized weekly with ammonium phosphate (16-20-0) and urea (46-0-0.) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water and adjusted based on SDVD.

Differences in growth performance, survival rate and feed consumption were statistically analyzed by ANOVA and the overall production costs were estimated to assess economic feasibility.

RESULTS AND DISCUSSION

Study 1 - Evaluation of 67% subsatiation daily feeding combined with 100% alternate day or 50% daily feeding versus continuous 67% daily feeding on growout of Nile tilapia in earthen ponds[1]

Figure 1 shows the average growth trend of stocks after 120 days of culture period in fertilized ponds. Average body weight of Nile tilapia on the 67%-50% or 67%-alternate day feeding did not differ significantly from animals fed at 67% subsation throughout the study.

Table 1 summarizes the growth performance, survival, feed consumption and yield of Nile tilapia cultured in earthen ponds using combined feed reduction strategies. Feed consumption per hectare was highest in fish fed 67% level (5201.1 $\pm$ 1238 kg/ha) compared with those animals fed at 67%-50% level (3965.2 $\pm$ 1037 kg/ha) and 67%-alternate day protocol (4045.3 $\pm$ 1104 kg/ha) Treatment III then Treatment II with a mean values of, 4,045.3 and 3,965.2 kgs hectare^{-1}, respectively. Fish fed daily at the 67% level had an FCR of 1.8 which was lower than that seen with fish on the 67%-50% and 67%-alternate day feeding protocol that had an FCR of 2.0. Despite these trends, there were no significant difference in feed consumed or FCR among treatments.

In terms of fish yield, the highest yield per hectare was observed in fish fed daily at 67% level, followed by those on the 67%-alternate day schedule, and then those on the 67%-50% protocol, with a mean values of 2,968.7, 2,024.7 and 1,980.7 kgs per hectare, respectively. Fish yield was signficantly higher in the 67% level group relative to other treatments ($P < 0.05$; Table 1).

The highest survival of 46.9% was found for fish fed at the 67% level relative to the other groups whose mean survival ranged 27.7-29.3%. There was some variation in survivorship of replicate groups fed at the 67% level and hence no statistically signficant difference was observed between this and the other groups. It is likely that the higher yield of fish on the 67% daily feeding regimen s is due to the elevated survivorship of this group relative to the other treatments. Generally, the survival obtained after the experiment was low due to observed mortality during the third and fourth month of the study. Recorded high water temperatures during the afternoon could have caused stress which affected growth and survival even with replenishment of water. This may have been exaserbated in those groups where fish were fed daily at either the 50% level or on alternate days at full levels.

Results on average minimum and maximum reading for water quality parameters during the 120-day culture period are summarized in Table 2. Generally, the dissolved oxygen readings during the morning ranged from 0.98 and 7.78 mg-L^{-1} for Treatment I, 1.20 and 3.89 mg-L^{-1} for Treatment II and 1.39 and 6.64 mg-L^{-1} for Treatment III. Afternoon dissolved oxygen readings for Treatments I, II and III were 3.64 and 12.87, 4.67 and 11.41 and 5.36 and 13.67 mg-L^{-1}, respectively. Dissolved oxygen concentration measured during the study remained in the favorable range for tilapia (Boyd 1990). The results support the findings of Liti et al. (2002) that Nile tilapia can tolerate low DO levels.

[1] The initial growout trial, e.g. Study 1, proposed for this investigation was initiated to assess the effect of high and lower dietary crude protein on growth performance of Nile tilapia in fertilized and nonfertilized ponds. Fry were sex reversed and fingerlings were stocked in ponds. However, due to production constraints, our industry cooperator (Santeh feed company of the Philippines) could not deliver the formulated feeds in adequate time to complete the study that was already in progress. Therefore, we improvised with the experiment already in progress by conducting Study 1 as outlined in this report., We evaluated the effects of a combined feed reduction on growout of tilapia using commercial feed in fertilized ponds. Also, our proposed second study (Study 2) essentially evaluates high and low dietary crude protein (with and without fishmeal) in fertilized ponds, hence it overlaps signficiantly with the original proposed Study 1, with exception that we did not test diets in unfertilized ponds. A majority of farmers fertilize there ponds so we have largely captured the objective of the original planned Study 1 through experiments outlined here with Study 2.

The average water temperature readings in the morning range between 28.43 and 31.97 ^{o}C, while afternoon water temperature ranged from 31.57 to 37.50 ^{o}C. Preferred water temperatures for tilapia growth are approximately 28.0-32.0 ^{o}C, but range varies depending on the tilapia species cultured. Tilapias reportedly tolerate temperatures up to 40 ^{o}C, but stress-induced disease and mortality are problematic when temperatures are around 37.0 or 38.0 ^{o}C (Teichert-Coddington et al. 1997). Fluctuation of the water temperature also affects growth due to the rise and fall of temperature, energy required for maintenance increases rapidly, thus decreasing the energy available for growth (Soderberg 1997).

Average pH ranged between 6.93 and 8.37. Boyd (1998) reported that waters with a pH range of 6.5 – 9 are the most suitable for fish production. Readings for total ammonia nitrogen and nitrite levels during the experiment were in the desirable range for all the treatments. The European Inland Fisheries Advisory Commission (1993) reported that the toxic level of NH_4 to fish is 2 mg/L. The average value of Secchi disc reading were 23.3 and 72.7, 22.3 and 78.3 and 24.3 and 57.7 cm for Treatments I, II and III, respectively. Water quality parameters showed no significant difference among treatments at 5% level of significance.

In our previous work we found that 67% daily feeding, 50% daily feeding and alternate day feeding at 100% level when used alone are all effective in reducing feed and production costs with little impact on total yield (Brown et al. 2000, Bolivar et al. 2003, Bolivar et al. 2006; Bolivar et al. 2010). With the 67% daily feeding protocol animals grew to virtually identical sizes at harvest relative to the 100% daily feeding level, while the other feed reduction protocols elicited up to 50% cost savings, but final size at harvest was slightly less, albeit insignificant relative to animals on a typical feeding regimen. The results presented here shows that a combined feed reduction strategy that incorporates a sequential series of a 60-day, 67% feeding rate with a 60-day 50% daily or 100% alternate day feeding rate is less effective than 67% feeding rate used alone in the pond growout of tilapia. Despite similar growth rates the survivorship of fish, and hence final extrapolated yield was reduced when using combined feed reduction strategies. We similarly found that the delayed onset of feeding combined with alternate day and 67% feeding rates was also less effective than that of animals fed at the typical 100% daily feeding rate (Borski et al. 2010; Bolivar et al. 2010). Collectively these results suggest that combined feed reduction strategies, at least based on the experimental paradigm tested here and previously, are less effective than applying 67% satiation, 50% satiation or 100% alternate day feeding strategies alone.

Study 2 – Evaluation of pond growout performance of Nile tilapia fed high and low crude protein diets with and without fishmeal.

The ingredients and proximate composition of the experimental diets are shown in Table 3. The 31% CP fishmeal and fishmeal free diets followed formulations from our previous work that showed the fishmeal free diets were 8% more cost effective than the standard diet containing fishmeal (Borski et al. 2011). Here we assess if reducing the crude protein levels of the diets might offer an additional cost benefit. Figure 2 shows the growth trend on average weight of Nile tilapia fed on four different diets after 120 days of culture period. The highest growth performance measured by final average weight of 287.722 g, final average length of 23.3 cm and specific growth rate of 6.227% were attained in tilapia fed 31% CP extruded feed without dietary inclusion of fishmeal (Table 4). The next best performance was seen in tilapia fed the 26% CP extruded feed without dietary inclusion of fishmeal where a final mean body weight, body length and specific growth rate was 269.226 g, 22.7 cm, and 6.169%, respectively. Fish fed the 31% CP extruded feed with fishmeal attained an average final body weight of 254.416 g, final length of 22.3 cm, and specific growth rate of 6.118%. Collectively, there were no significant differences in growth performance of fish fed the different diets ($P > 0.05$).

Similarly there was no significant difference in extrapolated yield of fish at harvest or in overall feed conversion ratio among the groups, although fish on the 26% CP, fishmeal free diet had the lowest FCR of 2.8 and highest extrapolated yield per hectare at harvest (Table 4). Low survival was partly due to the

presence of predatory bird throughout the culture period. Additionally exposure to suboptimal, cooler temperature (22 C) along with extreme temperature shifts (22 - 28 C) during the culture period may have contributed to poorer survival (Figure 3). Other farmers in the region also exhibited generally low survival during the experimental period. Rakocy and McGinty (1989) indicated that the preferred temperature range for better tilapia growth and survival is 28-30 ^{o}C.

Table 5 presents the minimum and maximum mean values of water quality parameters measured in each treatment over the course of the 120-day growth trial in ponds. Almost similar readings were observed for water quality parameters such as dissolved oxygen, water temperature, pH, alkalinity, total ammonia nitrogen, total phosphorus, CO_2, total hardness and Secchi disc visibility. All water quality parameter readings during the experiment fall under the tolerable range for tilapia culture.

In terms of cost and return analysis, all treatments had negative returns due to low number of stocks recovered during harvest (data not shown). Nevertheless, it is clear elimination of fishmeal and reducing the amount of crude protein in the diet provides a significant costs savings in feeds, which constitutes 60-70% of total production costs for growout of tilapia. The costs of feeds (43 PhP = $1) used in the experiments were as follows: PhP30.75 kg^{-1} for 31% CP with 6% fishmeal (treatment 1), PhP29.00 kg^{-1} for 26% CP + AA with fishmeal (treatment 2), PhP29.65 kg^{-1} for 31% CP with 0% fishmeal (treatment 3), and PhP27.20 kg^{-1} for 26% CP + AA with 0% fishmeal (treatment 4). With reference to the standard 31% CP with 6% fishmeal diet, the 26% CP with fishmeal diet costs 5.69%, the 31% CP with 0% fishmeal costs 3.58%, and the 26% CP with 0% fishmeal costs 11.54% less per kg than the standard diet.

Collectively, the results suggest and confirms our earlier report (Borski et al. 2011) that elimination of fishmeal from a standard 31% CP diet can produce a significant costs savings in feeds with no appreciable effect on growth performance and yield of tilapia raised in ponds. Here, we show that reductions in the amount of dietary CP, so long as the amino acid composition remains similar, can produce additional cost savings in feed of up to a 5.69% if fishmeal is included in the diet or up to 11.69% if fishmeal is not included in the lower protein diets. Hence, this work demonstrates that both elimination of fishmeal and reductions in the amount of crude protein or both significantly reduces the costs of feeds for growout of tilapia with little overall impact on growth parameters, survival or yield of fish.

Study 3 – Evaluation of dietary inclusion of nutritive protein binders and pellet processing aids on pellet durability and water stability of tilapia feeds.

The majority of the tilapia feed sold to farmers is in the form of extruded pellets, which increases the cost of the feed. The development of a pellet that is manufactured with a ring die pellet mill would reduce the manufacturing cost of the diets sold to farmers. Also, the number of manufacturers of aquatic feeds could be expanded if semi-sinking pelleted fish feeds rather than extruded feeds could be used, since many feed mills that currently produce pelleted feeds for livestock do not have costly extruders. The objective of this experiment was to identify pellet binders that improve the stability of pellets fed in ponds as an alternative to extruded tilapia feed. Two experiments were conducted to determine if the inclusion of nutritive protein binders and pellet processing aids would increase the durability and water stability of tilapia feed.

The average particle size of the basal diet (formulation in Table 6) was 291 and 281 microns in experiment 1 and 2, respectively. Based on traditional pellet durability tests The pellet quality results in both experiment indicated that that all pellets produced during the pelleting process were high quality (> 96% durability). The high quality pellets were primarily a result of the fine grinding of the basal diet, which increased the relative surface area contact between particles within the pellet. There was no difference in pellet durability index (PDI) between the control (no binder) diet and the diets containing the different binders (Table 7 and 8) based on traditional evaluation methods with the exception of the NHPT 90 sec test. Water stability testing was the primary method that produced significant differences between

the different pellet binders. The urea formaldehyde and wheat gluten treatments produced the best pellet water stability (PWS) results in Experiment 1. The urea-formaldehyde, gelatin, and wheat gluten increased (P <. 001) PWS by 37.3%, 5.7%, and 19.7%, respectively, over the no binder control (yielding 50.1% PWS). The pellets produced with urea-formaldehyde and wheat gluten had over 60% of the pellets intact at the end of the test. In experiment 2, the urea-formaldehyde, gluten, and urea-formaldehyde + gluten binders significantly increased (P < 0.05) PDI by 1.6%, 2.0%, and 2.2%, respectively, over the no binder control (91.5% NHPT PDI 90 sec test). The urea-formaldehyde, gluten and urea-formaldehyde + gluten binders increased (P < 0.001) PWS by 10%, 7%, and 15%, respectively over the no binder control and wheat gluten binder treatments (averaging 68% PWS). Although there was not statistical difference between the urea-formaldehyde as compared to the combination of 2% urea formaldehyde and 1% gelatin, the addition of the gelatin increased the water stability by 4.3%. In both studies, gelatin had a negative effect on pellet mill performance, causing the mill to choke a few times until the optimal feeder rate of the pellet mill was achieved. Based on these results, the combination of urea-formaldehyde and gelatin binders was included in commercial diets used in the pond research studies described below.

Study 4: Compare the effect on tilapia feed manufactured by extrusion and pellet processing on grow-out performance of Nile tilapia.

Our previous work (Borski et al. 2011) and results of Study 2 above, indicate fishmeal-free diets with 31% or 26% CP do not appreciably alter growth performance or yield of tilapia, but are more cost effective compared with standard diets containing fishmeal. Based on this information we compared extruded versus slow-sinking pelleted fishmeal-free diets that contain either 31% or 26% crude protein. Slow-sinking pelleted feeds contained 0.2% urea formaldehyde and 0.1% gelatin binder to improve pellet water stability (see Study 3 above).

As with the other pond study described in Study 2 we worked with our industry cooperator, Santeh Feed Corporation (Philippines), to develop the diets (see Table 9). This insured that least cost forumulated diets would incorporate ingredients widely available in the Philippines, including rice bran, copra cake, cassava meal, local fish oils and coconut oil.

Growth performance of Nile tilapia over the 135-day of culture period in earthen ponds is shown in Figure 4 and Table 10. Fish were provided the grower treatment diets during the last 60 days of growout. Figure 4 shows the growth pattern of the fish stocks prior to and during different dietary treatments. Fish fed the extruded 26% CP diet attained the highest final average body weight of 237.46 g, followed by those provided the pelleted 31% CP diet (227.95 g; P < 0.05). Both these groups had higher final average body weights and then those given the 31% extruded feed (180.67 g; P< 0.05). Fish fed the pelleted 26% CP diet had the the lowest final average body weight of 136.637 g that differed significantly from animals fed all other dietary treatments (P < 0.05). We found that these differences in growth began to diverge at day 45, which was prior to the onset applying the experimental diets at day 75. The difference in growth persisted until the end of the culture period. A similar pattern in overall daily weight gain and specific growth rate among the treatment groups was observed, although these difference were not statistically significant.

There were no differences in FCR or feed efficiency among the treatment groups. Fish provided the 31% CP pelleted feed had the highest yield with 3,288.7 kg/ha. This was followed by animals fed 31% CP extruded diets (3,214 kg/ha), and those fed 26% CP extruded feed (3,026.7 kg/ha). Animals fed 26% CP pelleted feed had the lowest extrapolated yield of 2,650.7 kg/ha. Overall, there was no significiant differences in yield among the treatment groups. Likewise, the survival of fish ranged from 42-51%, with lowest survival in fish fed the extruded 26% CP diet. Again survival rates among the groups were not signficantly different.

Table 11 shows the average reading of the water quality parameters monitored in this study. All water quality parameters recorded were within the ideal ranges suitable for the culture of tilapia and did not show variances among treatments that could have affected the performance of the fish stock.

The costs of fishmeal-free feeds (43 PhP = $1) used in the experiments were as follows: PhP30.20 kg^{-1} for extruded 31% CP diet (treatment I), PhP29.20 kg^{-1} for slow-sinking pelleted 31% CP (treatment II), PhP29.00 kg^{-1} for extruded 26% CP (treatment III), and PhP28.00 kg^{-1} for slow-sinking pelleted 26% CP feed (treatment IV). With reference to the standard extruded 31% CP diet, the 31% CP pelleted diet is 3.33%, the extruded 26% CP 4.00%, and the slow-sinking 26% CP diet is 6.67% less per kg. Collectively, these studies suggest that fishmeal-free extruded low CP and high CP diets produce similar performance with regard to growth, yield and survival of Nile tilapia, confirming results of Study 2. The use of high CP slow-sinking pelleted feed had the advantage over extruded feeds insofar as the cost of feed is 3.3% cheaper and performance is similar. It appears that sinking pelleted feeds at the lower crude protein level were somewhat less effective than the extruded feed, although no signficant loss of yield was observed with this feed compared with the others. Overall, it would appear that the use of sinking-pelleted feeds may have a cost effective advantage in tilapia production, particularly at the higher protein level. The results also suggest that manufacturers do not require costly extruders to produce effective tilapia aquafeeds.

CONCLUSION

It is estimated that 60-80% of total variable costs for growing tilapia is attributable to feeds. Through a series of studies we have established that feed costs can be reduced through modification of diet formulation and manufacturing with no significant impact on performance or yield of tilapia grown in ponds. The results suggest that elimination of fishmeal from a standard 31% CP diet can produce a significant costs savings in feeds with no appreciable effect on growth performance and yield of tilapia raised in ponds. This confirms our earlier report whereby fishmeal-free diet provide an additional 8% cost savings in growout of tilapia over and above the > 50% savings seen with an alternate day feeding protocol (Ayoola 2010; Borski et al. 2011). Additionally, we show that a reduction of dietary CP to 26%, so long as the amino acid composition remains similar to that of the higher 31% CP feed, can produce additional cost savings in feed of up to a 5.69% if fishmeal is included in the diet or up to 11.69% if fishmeal is not included in the lower protein diets. Hence, this work demonstrates that both elimination of fishmeal and reductions in the amount of crude protein or both significantly reduces the costs of feeds for growout of tilapia with little overall impact on growth parameters, survival or yield of fish.

Toward development of a less costly slow-sinking pelleted versus extruded feed we evaluated dietary binders in tilapia diets. The combination of urea-formaldehyde and gelatin were the most effective binders that maximized water stability of pelleted tilapia feeds. pelleted fishmeal-free diet over an extruded form is further effective in reducing feed costs by 3-7% with negligible impact on performance. This was particularly true of the higher 31% CP diet. The ability to produce pelleted fishmeal-free aquafeeds that are of comparable performance as extruded feeds can not only promote an additional cost savings in feed, but would allow manufacturers who lack costly extruders, which include most livestock feed companies, to expand to production of aquafeeds. Training on feed reduction strategies, least cost feed formulations and feed manufacturing technologies was provided to tilapia farmers, feed companies, government officials and the academic community through a workshop held in Batangas, Philippines, a central region of tilapia production in the Philippines.

LITERATURE CITED

Asian Development Bank. 2005. An evaluation of small-scale freshwater rural aquaculture development for poverty reduction. 163 pp

Axe, D. 2002. Feed production and technology manual. IMC Feed Ingredients. Lake Forest, IL.

Ayoola, A. 2010. Replacement of Fishmeal with Alternative Protein Source in Aquaculture Diets. M.S. Thesis. North Carolina State University. 129 pp.

Bolivar, R.B., Jimenez, E.B.J. and Brown, C.L. 2006. Alternate day feeding strategy for Nile tilapia grow out in the Philippines: Marginal cost-revenue analysis. North American Journal of Aquaculture, 68: 192-197.

Bolivar, R.B., C.L. Brown and E.T. Jimenez. 2003. Feeding Strategies to Optimize Tilapia Production in Ponds. Book of Abstract. Aquaculture 2003. Louisville, Kentucky, USA. p. 26.

Bolivar R.B., Vera Cruz E.M, Jimenez E.B.T, Sayco RMV, Argueza R.L.B, Ferket P.R., Stark C.R., Malheiros R, Ayoola A.A., Johnstone W.M., Picha M.E., Holler B.L., Won E.T., and R.J. Borski. 2010. Feeding reduction strategies and alternative feeds to reduce production costs of tilapia culture. Technical Reports: Investigations 2007-2009, AquaFish Collaborative Research Support Program. Oregon State University. Vol 1. p 50-78. (309 pp)

Borski, R. J., Bolivar, R. B., Jimenez, E. B. T., Sayco, R. M. V. and Argueza, R. L. B. 2010. Growth Performance of Nile Tilapia (*Oreochromis niloticus* L.) in Ponds in the Philippines using Combined Feed Reduction Strategies. Aquaculture 2010 Book of Abstracts. San Diego California, USA.

Borski, R.J., Bolivar, R.B., Jimenez, E.B.T., Sayco, R.M.V., Arueza, R.L.B., Stark, C.R., and Ferket, P.R. 2011. Fishmeal-free diets improve the cost effectiveness of culturing Nile tilapia (*Oreochromis niloticus, L.*) in ponds under an alternate day feeding strategy. p 95-101. *In* Liping L. and and Fitzsimmons K. (eds.). Proceedings of the Ninth International Symposium on Tilapia in Aquaculture. April 21-24. Shanghai, China. 407 p

Boyd, C. E. 1990. Water Quality in Ponds for Aquaculture. Alabama Agriculture Experiment Station, Auburn University, Alabama, USA.

Boyd, C. E. 1998. Water Quality for Pond Aquaculture. Research and development series No. 43. pp. 37. International Centre for aquaculture and aquatic Environments. Alabama Agricultural Experiment Station. Auburn University.

Brown, C.L., Bolivar, R.B., Jimenez, E. T., and Szyper, J.P. 2000. Timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds. p. 237-240. *In:* Fitzsimmons, K. and Filho, J.C. (eds.). Tilapia Aquaculture in the 21st Century. Proceedings from the Fifth International Symposium on Tilapia Aquaculture. September 3-7. Rio de Janeiro, Brazil. 682 p.

Brown, E.E. 1983. World Fish Farming: Cultivation and Economics. 2nd edn. AVI Publishing, Westport, CT. 397 pp.

Duncan, D. B. 1955. Multiple ranges and multiple F-tests. Biometrics, 11: 1-42.

El-Sayed, A.F.M. 2006. Nutrition and Feeding. In: Tilapia Culture. CABI Publishing, Oxford, U.K. 277 pp.

European Inland Fisheries Advisory Commission. 1993. Water quality criteria for European fresh water fish. Report on Ammonia and Inland Fisheries. Water Res., 7: 1011.

Ferket, P.R., E. van Heugten, T. A. van Kempen, and R. Angel, 2003. Nutritional Strategies to Reduce Environmental Emissions from Non-Ruminants. J. An. Sci. 80(Electronic Supplement 2): 2289 http://www.asas.org/jas/symposia/esupp2/default.asp

Jackson, A.J., B.S. Caper, A.J. Matty. 1982. Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. Aquaculture 27:97-109.

Leonard, G.O., S. Divakaran, A.G. Tacon. 2002. Methods for determining the physical stability of shrimp in water. Aquaculture Research, 33, pp 369-377.

Li, M.H., Lim, C.E., and Webster, C.D. 2006. Feed Formulation and Manufacture. In: Tilapia: Biology, culture and nutrition. C.E. Lim and C.D. Webster (Eds). The Haworth Press, Inc., New York. 517-559.

Lim, C.E. and Webster,C.D. 2006. Nutrient Requirement. In: Tilapia: Biology, culture and nutrition. C.E. Lim and C.D. Webster (Eds). The Haworth Press, Inc., New York. 469-501.

Middleton, T.F, P.R. Ferket, L.C. Boyd, H.V. Daniels, and M.L Gallagher. 2001. An evaluation of co-extruded poultry silage and culled jewel sweet potatoes as a feed ingredient for hybrid Tilapia (*Oreochromis niloticus X O. mossambicus*). Aquaculture 198:269-280.

Liti, D. M., Mac'Were, O. E. and Veverica, K. L. 2002. Growth performance and economic benefits of *Oreochromis niloticus/Clarias gariepinus* polyculture fed on three supplementary feeds in fertilized tropical ponds. *In*: K. McElwee, K. Lewis, M. Nidiffer, and P. Buitrago (eds.). *Nineteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP*, Oregon State University, Corvallis, Oregon. pp. 11-16.

National Research Council. 1993. Nutrient Requirements of Fish. National Academic Press, Washington, DC.

Rokey, G. and G. Huber. 2005. Aquatic Feed: Extrusion processing for aquaculture feeds. Feed Technology V. E. Schofield, ed. American Feed Industry Assn. Arlington, VA. pp 292-293.

Soderberg, R. W. 1997. Factors affecting fish growth and production. p. 199-213. *In:* H. S. Egna and C. E. Boyd (eds.). Dynamics of Pond Aquaculture. CRC Press. New York, USA. 437 pp.

Teichert-Coddington, D. R., Popma, T. J. and Lovshin, L. L. 1997. Attributes of Tropical Pond-cultured Fish. p. 183-198. *In:* H. S. Egna and C. E. Boyd (eds.). Dynamics of Pond Aquaculture. CRC Press. New York, USA. 437 pp.

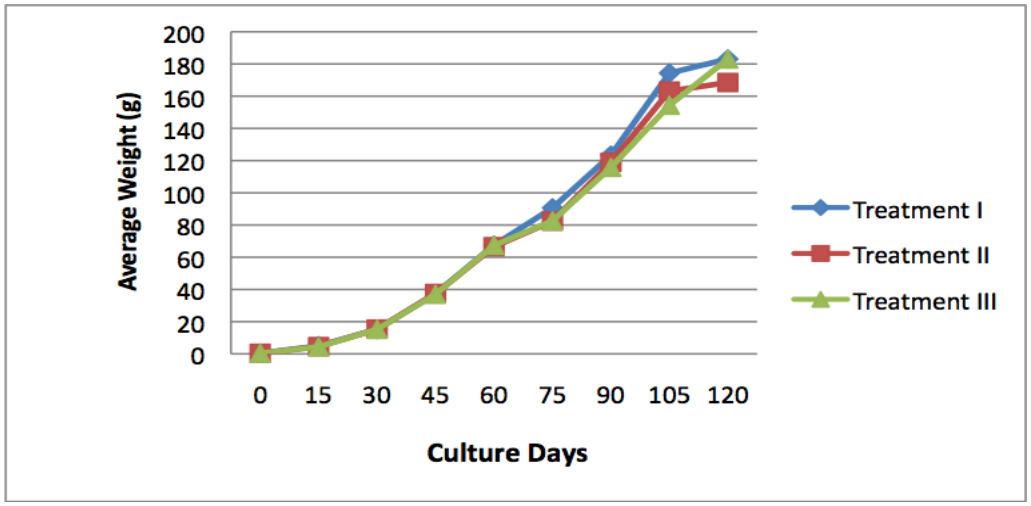


Figure 1. Average weight of Nile tilapia after 120 days of culture in ponds under combined feed reduction strategies: Treatment I – 67% daily feeding until harvest; Treatment II - 67% daily feeding for 60 days followed by 50% daily feeding until harvest; and Treatment III - 67% daily feeding for 60 days, followed by 100% alternate day feeding until harvest.

Table 1. Growth performance of Nile tilapia in ponds using combined feed reduction strategies.

Parameters	Treatments		
	67% daily feeding until harvest	**67% daily feeding for 60 days, 50% daily feeding until harvest**	**67% daily feeding for 60 days, 100% alternate day feeding until harvest**
Initial weight (g)	0.36 [a]	0.36 [a]	0.36 [a]
Final average weight (g)	183.1 ± 77.1 [a]	168.5 ± 39.9 [a]	183.1 ± 16.0 [a]
Initial length (cm)	2.8 [a]	2.8 [a]	2.8 [a]
Final average length (cm)	20.1± 2.9 [a]	19.9 ± 1.4 [a]	20.5 ± 0.6 [a]
Gain in weight (g)	182.7 ± 77.1 [a]	168.1 ± 39.9 [a]	182.7 ± 16.0 [a]
Daily gain in weight (g)	1.5 ± 0.6 [a]	1.4 ± 0.3 [a]	1.5 ± 0.1 [a]
Gain in length (cm)	17.3 ± 2.9 [a]	17.1 ± 1.4 [a]	17.7 ± 0.6 [a]
Daily gain in length (cm)	0.14 ± 0.02 [a]	0.14 ± 0.01 [a]	0.15 ± 0.00 [a]
Feed Conversion Ratio	1.8 ± 0.3 [a]	2.0 ± 0.1 [a]	2.0 ± 0.2 [a]
Yield per hectare (kg ha^{-1})	2968.7 ± 439.6 [a]	1980.7 ± 541.8 [b]	2024.7 ± 329.0 [b]
Feed consumed per hectare (kg ha^{-1})	5201.1 ± 1238 [a]	3965.2 ± 1037 [a]	4045.3 ± 1104 [a]
Survival (%)	46.9 ± 24.1 [a]	29.3 ± 4.7 [a]	27.7 ± 4.1 [a]

Means with the same letter superscipt are not significantly different ($P<0.05$).

Table 2. Average minimum and maximum reading for water quality parameters in ponds of Nile tilapia grown out for 120 days under combined feed reduction strategies.

Parameters	67% daily feeding until harvest		67% daily feeding for 60 days, 50% daily feeding until harvest		67% daily feeding for 60 days, 100% alternate day feeding until harvest	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**
Dissolved Oxygen (9AM) (mg-L^{-1})	0.98	7.78	1.20	3.89	1.39	6.64
Dissolved Oxygen (3PM) (mg-L^{-1})	3.64	12.87	4.67	11.41	5.36	13.67
Water Temperature (9AM) (^{o}C)	28.43	31.90	28.53	31.77	28.50	31.97
Water Temperature (3PM) (^{o}C)	31.70	36.37	31.57	36.27	32.00	37.50
Hydrogen-Ion (pH)	7.07	8.37	6.97	8.20	6.93	8.27
Total Ammonia Nitrogen (mg L^{-1})	0.017	1.090	0.018	1.456	0.022	0.942
Nitrite-Nitrogen (mg L^{-1})	0.067	0.075	0.067	0.075	0.075	0.075
Secchi Disc Visibility (cm)	23.3	72.7	22.3	78.3	24.3	57.7

Table 3. Ingredients (inclusion rate in kg ton^{-1} of feed) and proximate composition of caloric balanced extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids (AA) with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal).

RAW MATERIALS	31% CP with 6% fishmeal	26% CP + AA with 6% fishmeal	31% CP with 0% fishmeal	26% CP + AA with 0% fishmeal
Soybean Meal (HP) 45%	422.00	250.00	400.00	240.00
Corn Gluten	50.00	40.00	53.00	40.00
Hydrolyzed Animal Protein	30.00	30.00	30.00	30.00
Fishmeal Tuna 55%	60.00	60.00	0.00	0.00
Pork Meat Meal 55%	0.00	0.00	74.00	74.00
Copra Cake	73.00	111.20	76.00	135.00
Rice Bran	178.20	259.00	182.90	226.65
Cassava Meal	150.00	200.00	150.00	214.20
Fish Oil (Local)	5.50	5.00	5.00	5.00
Coconut Oil	5.00	15.00	5.00	10.00
Mono di-calcium phosphate	12.00	6.00	10.00	2.00
Salt	5.00	5.00	5.00	5.00
Mineral Premix	3.00	3.00	3.00	3.00
Vitamin Premix	6.30	5.80	6.10	5.80
L-Threonine	0.00	2.50	0.00	2.50
L-Lysine	0.00	6.00	0.00	5.25
DL-Methionine	0.00	1.50	0.00	1.60
TOTAL WEIGHT	**1000.00**	**1000.00**	**1000.00**	**1000.00**
DE Fish (kcal/kg)	2477.92	2455.75	2484.50	2443.86
Crude Protein (%)	30.99	25.71	31.07	25.66
Crude Fat (%)	6.21	6.87	6.23	6.67
Crude Fiber (%)	4.41	4.31	4.29	4.26
Starch (%)	17.36	22.33	17.40	22.57
Ash (%)	8.20	10.23	9.05	10.21
Ca (%)	0.96	0.94	0.91	0.92
Avail. Phosphorus (%)	0.67	0.64	0.66	0.64
Lysine (%)	1.51	1.50	1.50	1.49
Methionine (%)	0.54	0.54	0.50	0.53
Methionine+Cysteine (%)	0.97	0.87	0.96	0.89
Threonine (%)	1.06	1.05	1.07	1.07
Tryptophan (%)	0.33	0.25	0.42	0.34

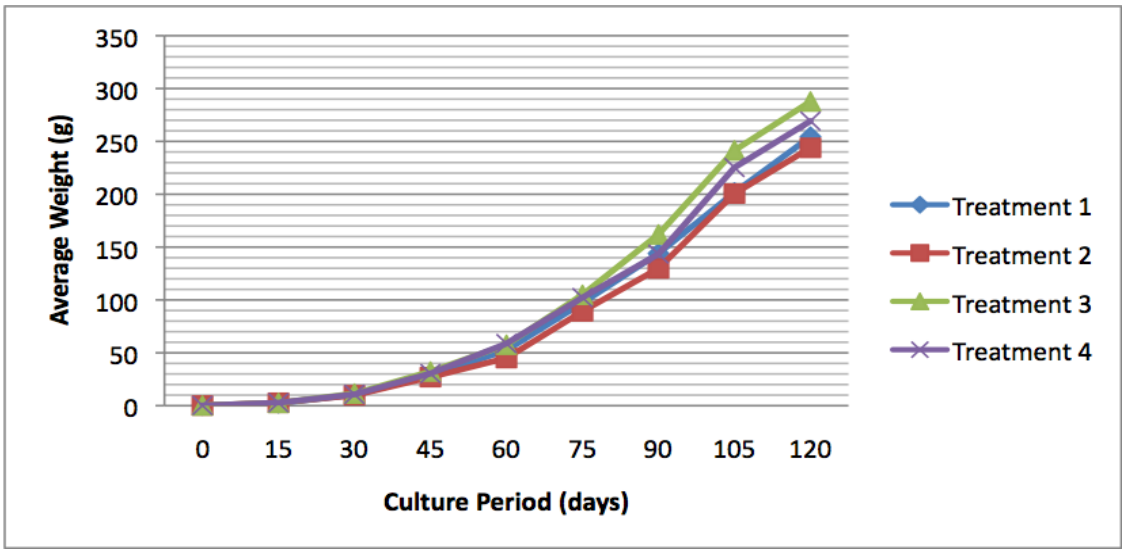


Figure 2. Average weight of Nile tilapia grown in ponds and fed extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal). Fish were fed different diets during the last 60 days of the 120-day culture period in ponds. Treatment 1 – 31% CP with 6% fishmeal; Treatment 2 - 26% CP supplemented with amino acids with 6% fishmeal; Treatment 3 - 31% CP extruded feed with 0% fishmeal; Treatment 4 - 26% CP supplemented with amino acids with 0% fishmeal. All diets were extruded and least cost formulated.

Table 4. Summary on growth performance, survival and yield of Nile tilapia fed extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids (AA) with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal). Fish were fed diets during the last 60 days of the 120-day culture period in earthen ponds.

PARAMETERS	**TREATMENTS**			
	31% CP with 6% fishmeal	**26% CP + AA with 6% fishmeal**	**31% CP with 0% fishmeal**	**26% CP + AA with 0% fishmeal**
Initial Average weight (g)	0.162 ± 0	0.162 ± 0	0.162 ± 0	0.162 ± 0
Final Average Weight (g)	254.416 ± 59.2	243.917 ± 12	287.722 ± 48.1	269.226 ± 50.8
Initial Average Length (cm)	2.337 ± 0	2.337 ± 0	2.337 ± 0	2.337 ± 0
Final Average Length (cm)	22.3 ± 1.3	22.1 ± 0.2	23.3 ± 1.2	22.7 ± 1.1
Gain in Weight (g)	254.254 ± 59.2	243.755 ± 12	287.560 ± 48.1	269.064 ± 50.8
Daily Gain in Weight (g)	2.119 ± 0.49	2.031 ± 0.10	2.396 ± 0.40	2.242 ± 0.42
Specific Growth Rate (%)	6.118 ± 0.19	6.097 ± 0.04	6.227 ± 0.15	6.169 ± 0.16
Gain in Length (cm)	20.0 ± 1.3	19.8 ± 0.2	21.0 ± 1.2	20.4 ± 1.1
Daily Gain in Length (cm)	0.167 ± 0.011	0.165 ± 0.002	0.175 ± 0.010	0.170 ± 0.009
Feed Conversion Ratio	3.4 ± 0.7	3.5 ± 0.9	3.2 ± 0.3	2.8 ± 0.7
Feed Conversion Efficiency	30.5 ± 6.7	30.3 ± 9.1	31.0 ± 2.5	37.0 ± 9.1
Yield per Hectare (kgs/ha)	2036.0 ± 501.8	1918.7 ± 452.6	2183.3 ± 242.7	2506.7 ± 691.1
Feed consumed per Hectare (kgs/ha)	6651.5 ± 624	6382.6 ± 555	7017.0 ± 210	6729.1 ± 355
Survival (%)	20.3 ± 6.6	20.6 ± 5.0	19.1 ± 2.3	23.3 ± 5.9

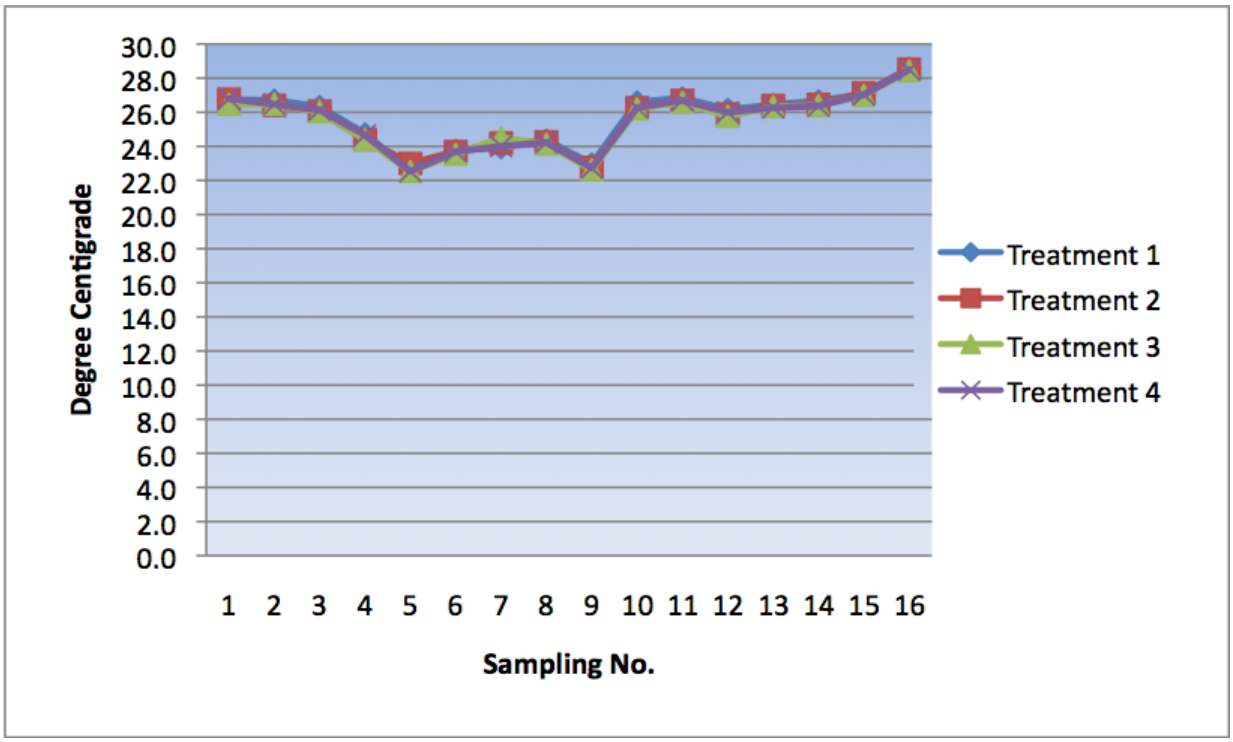


Figure 3. Weekly pond temperatures of Nile tilapia fed extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal). Fish were fed different diets during the last 60 days of the 120-day culture period in ponds. Treatment 1 – 31% CP with 6% fishmeal; Treatment 2 - 26% CP supplemented with amino acids with 6% fishmeal; Treatment 3 - 31% CP extruded feed with 0% fishmeal; Treatment 4 - 26% CP supplemented with amino acids with 0% fishmeal.

Table 5. Minimum and maximum mean values of pond water quality parameters measured over a 120 day culture period in which fish were fed extruded grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids (AA) with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal). Fish were fed diets during the last 60 days of the 120-day culture period in earthen ponds.

PARAMETERS	TREATMENTS			
	31% CP with 6% fishmeal	**26% CP + AA with 6% fishmeal**	**31% CP with 0% fishmeal**	**26% CP + AA with 0% fishmeal**
Dissolved Oxygen (mg- l^{-1})	1.98 – 6.10	2.09 – 6.25	2.03 – 6.39	2.98 – 6.36
Water Temperature (^{o}C)	22.57 – 28.57	22.77 – 28.53	22.53 – 28.43	22.53 – 28.50
Hydrogen Ion Concentration (pH)	6.87 – 8.43	6.57 – 8.63	7.27 – 8.63	7.37 – 8.67
Alkalinity (mg- l^{-1})	172.67 – 251.00	169.33 – 248.33	190.67 – 284.00	171.00 – 243.00
Total Ammonia Nitrogen (mg- l^{-1})	0.045 – 0.827	0.031 – 0.817	0.047 – 0.876	0.045 – 0.846
Total Phosphorus (mg- l^{-1})	0.287 – 0.498	0.210 – 0.493	0.292 – 0.502	0.237 – 0.520
Carbon Dioxide Concentration (mg- l^{-1})	0.00 – 36.33	3.67 – 35.00	2.67 – 34.67	0.00 – 24.67
Total Hardness (mg- l^{-1})	122.67 – 146.67	126.67 – 142.00	141.33 – 171.33	113.33 – 132.00
Secchi Disc Visibility (cm)	24.33 – 43.00	21.00 – 33.67	16.67 – 32.00	15.33 – 45.33

Table 6. The composition of the basal tilapia grower diet used to assess inclusion of nutritive protein binders on pellet durability and water stability**.** CP, crude protein.

Ingredient	**%**	**Calculated analysis**	
Corn	33.75	ME, kcal/kg	2650
Soybean meal (48 % CP)	25.00	Protein, %	25.50
Distillers Dried Grains w/ sol.	5.00	Ca, %	0.90
Rice bran	22.00	Available P, %	0.62
Poultry by-product meal, 65% CP	10.00	Total Lys, %	1.49
Poultry fat	0.50		
Limestone	0.45		
Dicalcium phosphate	1.55		
L-Lysine	0.30		
DL-Methionine	0.15		
Salt	0.50		
Vitamin premix	0.50		
Choline chloride (60%)	0.10		
Trace mineral premix	0.20		

Table 7. Pellet quality and water stability of tilapia feed with the addition of pellet binder.

Binder Treatment	NHPT 30[1]	NHPT 60[2]	PDI[2]	adjPDI[3]	PWS[4]	PCS[5]	MC[6]
	%						
Control	96.4	93.1	97.8	84.5	50.1[ab]	10.5	12.7
Urea-formaldehyde (0.2%)	96.9	94.0	97.8	86.8	68.8[d]	10.5	12.7
Gelatin (0.1%)	96.5	93.1	97.3[a]	85.6	53.0[ac]	10.0	12.6
Wheat Gluten (2.0%)	96.9	93.8	98.0	87.3	60.0[cd]	10.2	12.8
Carob Bean	97.0	93.9	97.7	85.2	48.8[a]	10.2	13.4[a]
SEM	0.15	0.27	0.14	0.67	3.00	0.28	0.17
Source of Variation P-value	0.0588	0.0550	0.0468	0.0810	0.0004	0.7259	0.0423

Table 8. Pellet quality and water stability of tilapia feed with the addition of pellet binder.

Binder Treatment	NHPT 30[1]	NHPT 60[1]	NHPT 90[1]	PDI[2]	MPDI[3]	PWS[4]	MC[6]
				%			
Control	96.8	94.2	91.5[a]	97.5	91.1	68.2[d]	11.47
Wheat Gluten (WG – 2%)	96.9	94.3	91.8[a]	96.7	91.7	67.7[d]	11.36
Gelatin (G - 0.1%)	97.2	94.8	93.3[b]	97.7	91.9	72.8[bc]	11.53
Urea formaldehyde (UF – 0.2%)[7]	97.0	95.3	93.0[ab]	97.4	92.6	75.1[ac]	11.50
UF(0.2%) + G(0.1%)	97.2	95.5	93.5[b]	97.8	92.9	78.4[a]	11.53
UF(0.2%) + WG (2%)	96.8	95.0	92.7[ab]	97.6	92.1	69.1[d]	11.93
SEM	0.28	0.30	0.41	0.22	0.43	1.11	0.11
Source of variation P-value	0.9023	0.0806	0.0441	0.1495	0.1428	0.0005	0.0423

[1]New Holmen Pellet Tester at 68 mbar and 30, 60, or 90 seconds.
[2]Pellet durability index, ASAE S269.3 Cubes, Pellets and Crumbles - Definitions and Methods for Determining Density, Durability, and Moisture Content.
[3]Modified pellet durability index ASAE S269.3 with the addition of three 19 mm hex nuts.
[4]Duplicate 50g samples of pellets from each treatment were place on a sieve with 3 mm diameter openings. The sample was slowly immersed in deionized water (24°C) for 10 min. The sieve was removed from water and drained for 1 min, oven-dried at 105°C for 9 h, cooled in a desiccator, and reweighed. Pellet water stability (PWS) was calculated as the percentage difference in sample weight after re-weighing and expressed as % of dry matter.
[5]Pellet compression strength, which was determined with a manually operated compression pellet tester (Amandas Kahl Nachf, 2057 Reinbek. Germany).
[6]Moisture content, American Association of Cereal Chemists. 1995. AACC Method 44-15A: Moisture—Air-Oven Method. In Approved Methods of the American Association of Analytical Chemists. Vol. 2. AACC, St. Paul, MN.
[7]Not approved for aquaculture feeds in the United States.

Table 9. Ingredients (inclusion rate in kg ton^{-1} of feed) and proximate composition of caloric balanced floating extruded and slow-sinking pelleted grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids (AA). All diets contained 0% fishmeal (fishmeal substituted with porkmeal). Slow-sinking pelleted grower test diets contained 0.2% urea-formaldehyde + 0.1% gelatin to enhance pellet water stability and durability.

RAW MATERIALS	Treatment I 31% CP floating extruded feed	Treatment II 31% CP slow-sinking pelleted feed	Treatment III 26% CP floating extruded feed	Treatment IV 26% CP slow-sinking pelleted feed
Binder (%) Urea-Formaldehyde	0.00	2.00	0.00	2.00
Binder (%) Gelatin Based	0.00	1.00	0.00	1.00
Soybean Meal (HP) 45%	400.00	400.00	240.00	240.00
Corn Gluten	53.00	53.00	40.00	40.00
Hydrolized Animal Protein	30.00	30.00	30.00	30.00
Fishmeal Tuna 55%	0.00	0.00	0.00	0.00
Pork Meat Meal 55%	74.00	74.00	74.00	74.00
Copra Cake	76.00	76.00	135.00	135.00
Rice Bran	182.90	179.90	226.65	223.65
Cassava Meal	150.00	150.00	214.20	214.20
Fish Oil (Local)	5.00	5.00	5.00	5.00
Coconut Oil	5.00	5.00	0.00	0.00
Mono di-calcium phosphate	10.00	10.00	10.00	10.00
Limestone	0.00	0.00	2.00	2.00
Salt	5.00	5.00	5.00	5.00
Mineral Premix	3.00	3.00	3.00	3.00
Vitamin Premix	6.10	6.10	5.80	5.80
L-Threonine	0.00	0.00	2.50	2.50
L-Lysine	0.00	0.00	5.25	5.25
DL-Methionine	0.00	0.00	1.60	1.60
TOTAL WEIGHT	**1000.00**	**1000.00**	**1000.00**	**1000.00**
DE Fish (kcal/kg)	2,484.50	2,486.94	2,443.86	2,443.41
Crude Protein (%)	31.00	31.00	26.00	26.00
Crude Fat (%)	6.23	6.22	6.67	6.67
Crude Fiber (%)	4.29	4.25	4.26	4.24
Starch (%)	17.40	17.32	22.57	21.86
Ash (%)	9.05	8.91	10.21	9.32
Ca (%)	0.91	0.91	0.92	0.92
Avail. Phosphorus (%)	0.66	0.66	0.64	0.64
Lysine (%)	1.50	1.49	1.49	1.49
Methionine (%)	0.50	0.50	0.53	0.53
Methionine+Cysteine (%)	0.96	0.93	0.89	0.88
Threonine (%)	1.07	1.07	1.07	1.07
Tryptophan (%)	0.42	0.42	0.34	0.34

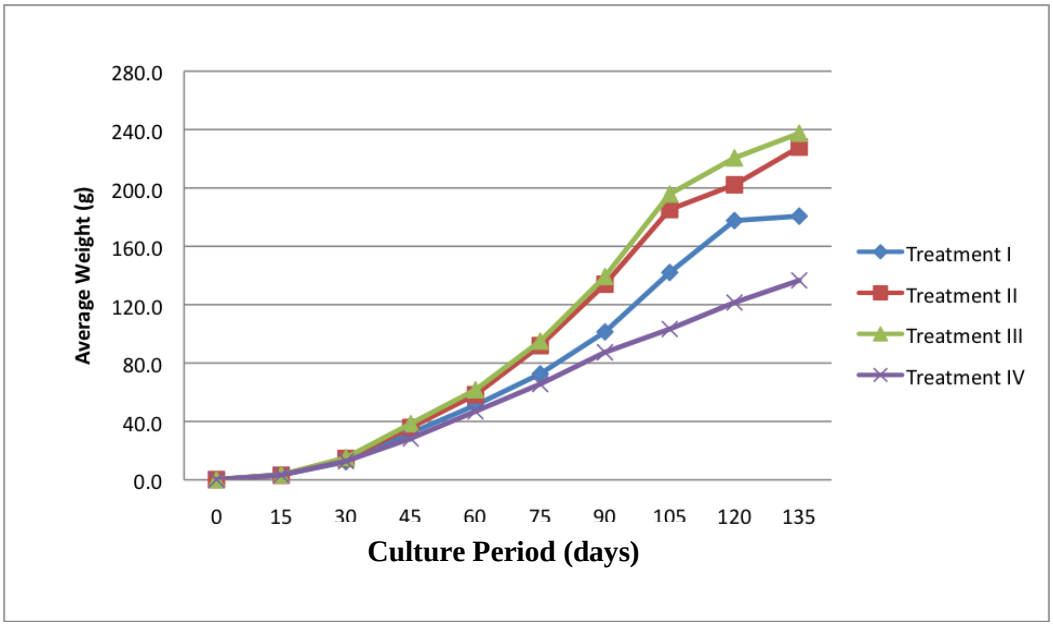


Figure 4. Average weight of Nile tilapia grown in ponds and fed floating extruded or slow-sinking pelleted, fishmeal-free grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids. Fish were fed different diets during the last 60 days of the 135-day culture period in ponds. Treatment 1 – 31% CP floating extruded feed; Treatment II - 31% CP slow-sinking pelleted feed; Treatment III - 26% CP floating extruded feed; Treatment IV - 26% CP slow-sinking pelleted feed. All diets were least cost formulated. Diets with 26% CP were supplemented with amino acids to provide similar amino acid profiles as the 31% CP diets. All slow-sinking pelleted diets contained 0.2% urea-formaldehyde and 0.1% gelatin to enhance pellet water stability.

Table 10. Average weight of Nile tilapia grown in ponds and fed floating extruded or slow-sinking pelleted, fishmeal-free grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids. Fish were grown for 135 days and treatment diets were applied from day 75 until harvest. mean ± SEM

PARAMETERS	Treatment I 31% CP floating extruded feed	Treatment II 31% CP slow-sinking pelleted feed	Treatment III 26% CP floating extruded feed	Treatment IV 26% CP slow-sinking pelleted feed
Initial Average Body Weight (g)	0.170 ± 0.0	0.170 ± 0.0	0.170 ± 0.0	0.170 ± 0.0
Final Average Body Weight (g)	180.667 ± 3.01[a]	227.947 ± 5.46[b]	237.457 ± 7.87[b]	136.637 ± 2.03[c]
Initial Average Total Length (cm)	2.288 ± 0.0	2.288 ± 0.0	2.288 ± 0.0	2.288 ± 0.0
Final Average Total Length (cm)	20.526 ± 0.60[a]	21.110 ± 0.15 [a]	21.033 ± 0.21[a]	18.160 ± 0.10 [b]
Daily Gain in Weight (g/day)	1.504 ± 0.14	1.898 ± 0.47	1.977 ± 0.72	1.137 ± 0.05
Daily gain in Total length (cm/day)	0.152 ± 0.01	0.157 ± 0.01	0.156 ± 0.02	0.132 ± 0.00
Specific Growth Rate (%)	5.800 ± 0.08	5.948 ± 0.21	5.932 ± 0.29	5.573 ± 0.04
Survival Rate (%)	48.4 ± 2.20	44.7 ± 11.6	42.1 ± 11.6	50.6 ± 3.9
Extrapolated Fish Yield per Hectare (kg/ha)	3214.0 ± 194.0	3288.7 ± 626.5	3026.7 ± 147.3	2650.7 ± 147.6
Extrapolated Feed Consumed per Hectare (kg/ha)	5986.6 ± 238.5	6007.7 ± 533.8	6062.1 ± 633.2	5167.0 ± 313.9
Feed Conversion Ratio	1.9 ± 0.05	1.9 ± 0.20	2.0 ± 0.12	2.0 ± 0.01
Feed Conversion Efficiency (%)	53.5 ± 1.3	53.8 ± 5.7	50.4 ± 3.0	51.2 ± 0.3

Table 11. Mean and range values for water quality parameters recorded weekly for Nile tilapia grown in ponds and fed floating extruded or slow-sinking pelleted, fishmeal-free grower test diets formulated with 31% crude protein (CP) or 26% CP supplemented with amino acids. Fish were grown for 135 days and treatment diets were applied from day 75 until harvest.

Parameters		Treatments			
		Treatment I 31% CP floating extruded feed	**Treatment II 31% CP slow-sinking pelleted feed**	**Treatment III 26% CP floating extruded feed**	**Treatment IV 26% CP slow-sinking pelleted feed**
DO (mg l^{-1})	Mean	3.53	3.86	3.84	3.70
	Min - Max	1.07-8.50	1.46-6.35	1.56-7.35	1.14-9.32
Temperature (^{o}C)	Mean	29.4	29.4	29.3	29.5
	Min - Max	24.8-31.7	24.8-31.8	24.6-31.5	25.0-31.7
pH	Mean	8.2	8.2	8.2	8.2
	Min - Max	7.2-9.0	7.1-9.2	7.4-9.3	7.3-9.3
Total Alkalinity (mg l^{-1})	Mean	200.5	211.9	202.0	201.4
	Min - Max	168.3-237.0	168.0-253.7	166.7-245.0	169.3-243.7
Total Ammonia Nitrogen (mg l^{-1})	Mean	0.090	0.094	0.084	0.083
	Min - Max	0.007-0.199	0.018-0.238	0.012-0.237	0.009-0.174
Total Phosphorus (mg l^{-1})	Mean	0.323	0.394	0.347	0.378
	Min - Max	0.120-0.685	0.173-0.675	0.142-0.628	0.200-0.677
Secchi Disc Visibility (cm)	Mean	25.9	24.5	26.2	24.2
	Min - Max	16.7-76.7	18.0-63.3	17.3-73.3	19.3-73.3

Develop Feeding Strategies for *Moringa Oleifera* and *Leucaena Leucocephala* as Protein Sources in Tilapia Diets

Sustainable Feed Technology/Experiment/09SFT05PU

Rebecca Lochmann, Carole Engle, and Tom Kasiga
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

Sebastian W. Chenyambuga, Hegi Shighulu, Nazael Madalla, and Berno V. Mnembuka
Sokoine University of Agriculture,
Morogoro, Tanzania

Kwamena Quagrainie
Purdue University
West Lafayette, Indiana, USA

ABSTRACT

Digestibility and feeding trials were performed to evaluate *Moringa oleifera* and *Leucaena leucocephala* leaf meals as protein sources (compared to soybean meal) in Nile tilapia diets. Both leaf meals were obtained from Tanzania. The leaf meals were soaked in water to reduce anti-nutritional factors, dried, and ground to a small particle size before incorporation into diets. Five diets were made for both trials: the control diet contained 50% soybean meal (SOY), and diets 15 MOR and 30 MOR were made by substituting 15 and 30% of the soybean protein with *Moringa* protein. Diets 15 LEU and 30 LEU were made by substituting 15 and 30% of the soybean protein with *Leucaena* protein. For the digestibility trial, diets containing 32% crude protein were used. Diets with 36% protein were used for the feeding trial due to the small initial fish size. The digestibility trial was carried out using mixed sex Nile tilapia of 200-400 g in 150-L indoor tanks in a recirculating system with dechlorinated municipal water maintained at 28^0C. Other water quality parameters were kept at optimum levels for Nile tilapia. Fish were conditioned to the experimental diets and the fecal removal process (fresh, floating feces were collected with a net) for 1 week. Fecal collection was conducted 8-10 hours after the last meal in the evening, as well as the next morning before feeding. The fecal samples were dried at 50^0C for 12 hours and then frozen until analysis. The apparent protein digestibility (APD, mean±SE, %) of the SOY diet (86.35±0.87) was higher than all others. The APD of the 15 MOR (84.69±0.59) and 30 MOR (83.34±1.10) diets were similar to each other and higher than the APD of the 15 LEU (78.49±0.91) and 30 LEU (74.70±0.52) diets. The APD of 30 LEU was also lower than that of the 15 LEU diet. Overall, leaf meals reduced protein digestibility compared to soybean meal, but diets containing *Moringa* were digested better than those containing *Leucaena*. There was no difference in the apparent lipid digestibility (%) of the SOY (95.83±0.34) and 15 MOR (95.19±0.62) diets. Lipid digestibility of the SOY diet was higher than that of 30 MOR (92.99±1.17), 15 LEU (94.46±0.06), and 30 LEU (92.98±0.24) diets. Lipid digestibility of the 30 MOR and 30 LEU diets was lower than that of the other diets. Both protein and lipid digestibility were inversely related to concentration of dietary fiber. For the feeding trial, 100 mixed-sex Nile tilapia, averaging 5.16 g individually were stocked in 1500-L outdoor circular plastic tanks supplied with reservoir water. The

tanks were maintained as static systems except for periodic flushing when water quality parameters fell below the optimum for Nile tilapia. Subsamples of 30-50 fish were weighed every two weeks and water quality parameters were monitored weekly. The trial was terminated after 60 days. There were no differences in growth, feed conversion, survival, lysozyme, or proteolytic enzyme activity among treatments. Proximate and fatty acid composition of whole fish is still in progress.

INTRODUCTION

Over the last 30 years, aquaculture has grown worldwide faster than any other animal production sector (FAO 2007a). The average annual growth rate of aquacultural production was 10% compared with 3% in the cattle industry and 1.6% in captured aquatic species from natural environments. The rapid rise in aquaculture has generated an increase in the production of aquatic animal feeds. The estimated increase in production of aquaculture feeds from 2000 to 2010 was from about 13 million metric tonnes (mmt) to 30 mmt (Francis et al. 2001). Securing raw materials for these feeds will be a continuous challenge for the aquaculture industry.

In Africa, economic studies have demonstrated that fish farming is a viable enterprise combining high gains with minimal costs (Molnar et al. 1991). Wijkstrom and MacPherson (1990) indicated that large-scale and intensive aquaculture enterprises are beyond the means of most farmers in Africa, but small-scale aquaculture with commercial orientation can be a profitable economic activity.

Some of the problems that make tilapia farming in Tanzania unprofitable include low productivity resulting from poor management, and low seed and feed quality (FAO 2006). Low productivity has a nutritional component due to the imbalance of energy and protein in fish diets commonly used by small-scale farmers. In Tanzania, farmers use naturally available feeds or feedstuffs in fish culture. Ponds are commonly fertilized with domesticated animal droppings or tender leaves as compost manure to stimulate plankton growth. The most frequently used feeds are rice and maize bran, kitchen leftovers, and garden remains. These are of low quality, and fish reared on these feeds are unable to meet their maintenance and production requirements, especially for protein. This prolongs the time to reach market weight and consequently leads to production of poor-quality fish and hence, low profitability of fish farming.

In formulation of fish feeds, the nutrient requirements of fish, as well as the nutrient composition of feedstuffs, price, palatability quality, and availability of various feedstuffs should be considered (De Silva and Anderson 1998). Protein is a critical component in complete fish diets and is always given a high priority in formulation of complete feeds. Protein inadequacy may lead to poor growth and generally inferior performance. Protein is also usually the most expensive component of fish feeds, accounting for more than 50% of total feed costs in intensive aquaculture (Thompson et al. 2005). In semi-intensive systems with abundant natural foods, the protein content of supplementary feeds may be reduced and the carbohydrate content increased (Hepher 1988).

Consequently, efforts have been shifted to evaluating potential alternative protein sources for use in fish diets. Alternative protein sources include soybean meals, protein concentrates (Refstie et al. 1998), meat meals (Williams et al. 2003) and blood meals (Bureau et al. 1999). Soybean meal has been the focus of attention for fish meal substitution because of its advantage in terms of protein quality, competitive price and adequate supply. However, soybean meals and agro-industrial by-products have not been readily

adopted by small-scale fish farmers in sub-Saharan Africa due to cost and limited supplies. There is a need to identify less expensive alternative sources of protein from locally available feed resources and to select protein sources that do not conflict with human food security interests (El-Sayed 1999; El-Saidy and Gaber 2002). For instance, fish meal, soybean meal, meat meal and blood meal are likely to be reserved for human rather than animal diets in Tanzania.

Leguminous tree leaves and their pods seem to be appropriate alternative protein sources to fish meal and soybean meal (Fernandes et al. 1999; El-Saidy and Gaber 2003; Kaushik et al. 2004). In particular *Leucaena leucocephala* (Pantastico and Baldia 1980; Ferraris et al. 1986; Santiago and Lovell 1988) and *Moringa oleifera* leaf meals (Richter et al. 2003; Afuang et al. 2003) may lower feed costs and increase the profitability of fish farming enterprises because leaf meals are cheaper than fish meal or soybean meal.

Moringa oleifera (hereafter referred to as *Moringa*) has positive agronomic attributes such as the ability to resist adverse soil and climatic conditions and yet sustain a reasonably high yield (Palada and Chang 2003). More than 11,300 hectares of *Moringa* trees have been planted in various parts of Tanzania, primarily for its oil seeds (Creighton, 2001). The potential of *Moringa oleifera* as an ingredient in fish feed formulations lies in its local availability, affordability and relatively good nutritional profile. Another advantage is its multiple uses, which potentially could serve as a source of additional income to the farmers. For instance, oil from *Moringa* seeds is used in perfumes and lubrication of fine machinery, while powder from the seed kernel has coagulant properties which can be used to clarify turbid water (Palada and Chang 2003).

The amino acid pattern of *Leucaena* (hereafter referred to as *Leucaena*) is comparable to that of soybean and fish meal (Ter' Meulen et al. 1979) or other animal feed sources available in developing countries (D'Mello and Thomas 1977; Ter' Meulen et al. 1979; Kale et al. 1987). The protein concentration in *Leucaena* is about 23.5-31.5% (Kimbi 1997; El-Hassan et al. 2000; Kimoro 2003). *Leucaena* is now being grown in most parts of Tanzania due to its outstanding nutritional value to ruminants ((1994). Overall, the information on the feeding value of *Leucaena* and *Moringa* is not conclusive; particularly, the digestibility and the effects of *Leucaena* and *Moringa* on overall performance and enzyme activity in Nile tilapia. Hence there is a need to find out the extent to which these leguminous tree leaves can replace fish meal or soybean meal as protein sources in small-scale tilapia production.

The greater abundance of vegetable products has attracted research interest as ingredients for fish feed production (El-Sayed 1999). The use of raw vegetables is limited, however, by their antinutritional factors (Table 2) which are grouped into three categories: (1) those affecting protein utilization and digestion; (2) those affecting mineral utilization; and (3) anti-vitamins and toxic substances (Francis et al. 2001). Higher replacement levels (>50%) of fish meal or soybean meal by plant ingredients can also reduce palatability (Hassan et al. 1997), nutrient utilisation (Eusebio et al. 2004), growth (Eusebio and Coloso 2000), or cause poor reproductive performance (Santiago and Lovell 1988).

In this study we hypothesize that the replacement of soybean meal with *Leucaena* leaf meal and *Moringa* leaf meal as protein sources in fish diets may lower feed costs and hence increase the profitability of fish

farming enterprises. The major objective of this study is to evaluate the feeding value of *Leucaena* and *Moringa* leaf meals as protein sources in Nile tilapia diets as assessed by the following sub-objectives:

1) To compare feeding levels at 5, 7.5, and 10% of body weight on growth performance and feed conversion efficiency of tilapia fed diets containing sunflower seed cake, Moringa oleifera and Leucaena leucocephala as protein sources (Sokoine University of Agriculture, Tanzania).
2) To compare alternate day feeding with daily feeding on growth performance and feed conversion efficiency of tilapia fed diets containing sunflower seed cake, Moringa oleifera and Leucaena leucocephala as protein sources (Sokoine University of Agriculture, Tanzania).
3) To evaluate the digestibility of *Moringa oleifera* and *Leucaena leucocephala* leaf meals using mixed sex Nile tilapia *Oreochromis niloticus* (University of Arkansas at Pine Bluff, USA).
4) To evaluate the effect of feeding *Moringa oleifera* and *Leucaena leucocephala* on growth performance, feed conversion ratio, survival, proximate and fatty acid composition of whole body, nonspecific immune responses, and proteolytic enzyme activity of mixed sex Nile tilapia (University of Arkansas at Pine Bluff, USA).

METHODS

Objectives 1 and 2

These objectives were being achieved from studies in Tanzania at Sokoine University of Agriculture (SUA). A preliminary study first developed ten different diets from three protein sources, i.e., soybean, sunflower seed cake and *Moringa* leaf (Table 1). The diets were then tested at different inclusion levels of the three protein sources. Preliminary results suggest Diet 3 (Table 1) performed better compared to the other diets, and consequently is being used in experiments to address objectives 2 & 3 in pond feeding trials using SUA's standard protocols. These experiments did not begin on schedule due to funding delays, and could not be conducted concurrently due to limitations on numbers of ponds available for the trials. The expected completion date for these objectives is April, 2012.

Objectives 3 & 4

The study consisted of two different trials: 1) A digestibility trial; and 2) A feeding trial. The digestibility trial was carried out in a recirculating system in indoor tanks in the UAPB fish nutrition wet lab, and the growth trial was conducted in a static system in outdoor tanks at the UAPB aquaculture research station. After the trials were completed, all live fish were returned to the UAPB aquaculture research station.

Source of fish

Oreochromis niloticus (2500) from stocks maintained at the UAPB aquaculture station were used for the digestibility study, and smaller fish for the growth trial were obtained from a commercial farm in Alabama.

Source and preparation of Leucaena and Moringa meals

The test ingredients (*Moringa* and *Leucaena*) were obtained from Tanzania. Prior to shipment to the US, Sebastian Chenyambuga (Soloine University, Tanzania) soaked the leaves in water to reduce antinutrients and sun-dried them. Upon arrival at UAPB, the leaf meals were soaked again (for 3 days at room temperature) to further reduce mimosine (Hassan et al. 1994; Wee and Wang 1987), a toxin in *Leucaena*, and saponins in Moringa (Tacon 1985). During soaking at UAPB, the leaf meal-water mixtures were stirred for 1 h daily. After three days of soaking, the mixtures were filtered through a 0.5-mm sieve. The

residue was fan-dried for 24 h and then lyophilized for 60 h using an MD3053 model freeze drier (Mill Rock Technologies., San Diego, California). The dried leaves of *Moringa* and *Leucaena* were then finely ground before incorporation into diets.

Proximate composition of Moringa and Leucaena meals

Protein, crude fiber, dry matter and ash content of the two leaf meals, formulated diets, feces, and whole fish from the feeding trial were analyzed according to Standard Methods (AOAC 1995). The Folch method (Folch et al. 1957) was used to analyze the total lipids. The Kjeldahl method (AOAC 1995) was used for crude protein, and the Ankom 200 fiber analyzer (Ankom Technology Corp., Fairport, New York) was used for crude fiber. Lipid extracts from the leaf meals, diets, and whole body will be used for fatty acid analysis (leaf meals and diets only). Fatty acid methyl esters (FAME) were analyzed (Morrison and Smith 1964) using a flame ionization gas chromatograph (Varian, Model CP-3800 fitted with a CP-8200 autosampler, Walnut Creek, CA) with helium as the carrier gas. The FAMEs were separated on a fused silica capillary column (15m x 0.25 mm ID; Varian CP select for Fame #CP8510). Injection volume was 1 µl, with an injector and detector temperature of 250°C and 315°C, respectively. The column temperature was held initially at 100°C for 10 min., increased to 160°C at a rate of 15°C/min. and held for 4 min., then increased to 250°C at a rate of 2.5°C/min. Each sample had a total analysis time of 60 min. The FAMEs were identified and quantified by comparing the retention time and peak area to those of serially diluted mixtures of reference standards (GLC-96, GLC-473b, Nu-Check Prep, Elysian, MN). The results of the individual fatty acids were expressed as g/100g of total identified FAMEs.

Diet composition

Five isonitrogenous (32% crude protein) and isocaloric diets (18 KJ/g) were formulated for the digestibility trial, whereas diets for the feeding trial contained 36% protein because smaller fish were used. The protein-to-energy ratio of the diets will be 100 mg of protein per Kcal of energy (Suresh 2003). The reference diet contained soybean meal as the primary protein source (Table 3). Four other diets contained the alternative protein sources *Leucaena* or *Moringa***.** These ingredients replaced soybean meal on an equal protein basis, at 15% or 30%. This resulted in a total of five diets: diet 1 was the reference diet, and 15 % and 30% of the protein in soybean meal in the reference diet was replaced with *Moringa* to make diets 2 and 3. Diets 4 and 5 were produced by replacing 15% and 30% of the protein in the soybean meal diet with the protein in *Leucaena*. Similar diet formulas (with the exception of protein amount) were used for both the digestibility and feeding trials. For the digestibility trial chromic oxide was added as an inert marker, and for the growth trial, chromic oxide was replaced by wheat middlings.

Preparation of diet

All ingredients were finely ground (1-2 mm) in a Wiley mill at UAPB prior to inclusion in diets. The diets were prepared in the fish nutrition lab at UAPB by slowly adding the micro-ingredients (vitamin and mineral premixes) to the macro-ingredients to ensure a homogenous mixture. Between 400 and 450 mL of distilled water was added per kilogram of diet to achieve a consistent mixture that produced stable pellets. A meat grinder fitted with 6.25- or 3.125-cm dies was used to produce different sized pellets, which were fan dried for 8 h and stored at -18^{o}C until use. The bigger pellets were used for the digestibility trial.

Digestibility trial

Mixed sex fish (N=10), weighing 200-400 g in individual weight were stocked in each 150-L tank. The gender of the fish was manually determined and a fixed gender ratio of 9 males to 1 female was used in

all tanks. Three replicate tanks were used per diet. Fish were maintained in a recirculating system supplied with dechlorinated municipal tap water. The temperature was maintained near 28˚C, which is considered the optimal temperature for growth for *Oreochromis niloticus* (Popma and Masser 1999). Temperature will be recorded daily. The flow rate in each tank was set at 1.1 L/min, which resulted in the total replacement of tank water volume every 2 hours to ensure proper clearance of ammonia. Each tank had individual water and air valves and was aerated by individual air stones. The water quality parameters monitored were pH (UB-10 pH/ mV meter, Denver Instruments, Colorado), total hardness (EDTA/ManVer method), dissolved oxygen (YSI 55, YSI Incorporated, Yellow springs, Ohio), and total ammonia nitrogen (TAN) (salicylate/cyanurate method, pH adjusted to 7, DR/890 colorimeter/high range Test'N® Tube, Hach Company, Loveland, Colorado). Equations from Emerson et al. (1975) were used to calculate the percentage of unionized ammonia from total ammonia. Calcium chloride was added to the water to maintain hardness $\geq$ 50 mg/L as calcium carbonate. The fish were fed their respective diets to apparent satiation once daily for 5-7 days prior to collection of feces to allow them to adjust to the diets.

Sampling feces

We attempted stripping feces from the fish, but they were apparently stressed by the procedure and refused feed for days after each stripping period. Therefore, evacuated feces were collected by siphoning each day for a week instead. Fecal collection was conducted 8-10 hours after the last meal in the evening, as well as the next morning before feeding. All feces collected from each tank were combined in the same aluminum pan and frozen between collections. Once sufficient feces were collected (>10grams per tank), the pans containing feces were dried in the oven at 50^0C for 12 hours, and then stored at -20^0C until analyzed.

Calculation of apparent digestibility

We assumed that there was no variation in the content of chromic oxide in the feces. Chromic oxide concentrations in the diets and the feces were determined by AOAC (1995) methods.

The concentration factor (CF) was determined by the method of Sugiura (2000):
CF= chromic oxide concentration in feces / chromic oxide concentration in diet.
The CF indicates a portion of the feces that corresponds to a unit amount of the diet, therefore the nutrient content in the feces was divided by the CF.
Digestibility (%) = 100X {nutrient concentration in the diet - Concentration of nutrients in feces/CF}/nutrient concentration in the diet.

Growth trial

Stocking density and feeding for the growth trial

The growth trial was conducted using mixed-sex fish, but the fish were too small to identify gender prior to stocking so fixed ratios of males and females could not be achieved. Individual initial mean weight of fish was 5.2$\pm$0.02. One hundred fish were stocked into 20 1500-L outdoor tanks (4 tanks per diet) in a static system supplied with reservoir water. Fish were fed twice daily to satiation for 60 days. Water quality parameters were monitored as described for the digestibility trial. In addition, chlorophyll a (chloroform–methanol extraction, Lloyd and Tucker 1988) was measured in the feeding trial. When water quality in any tank fell below acceptable limits for Nile tilapia, (> 0.05 ppm un-ionized ammonia, > 8 or < 6.5 pH), 50% of the water in all the pools was flushed with fresh water to restore the water quality to acceptable conditions. Each tank was filled separately with a tap and had an individual stand pipe for

drainage and air stone for aeration. Subsamples of fish were weighed every two weeks throughout the trial to assess growth and adjust feed rations. Feed intake and mortalities were recorded daily. The average protein deposition was calculated by determining the difference in the protein content of fish before and after the growth trial. The average protein intake was calculated as the dry protein in feed (g) in the feed consumed. At harvest, the overall growth, feed conversion, survival, non-specific immune function, digestive enzyme activity, and body composition were evaluated.

Determination of growth rate and individual weight gain
Growth rate = 100X (final weight-initial weight) / days in cycle.
The mean individual weight gain = mean final individual weight-mean initial individual weight.

Determination of feed conversion ratio (FCR)
FCR= Feed consumed (g of dry matter) / live weight gain (g).

Determination of apparent net protein utilization (ANPU)
ANPU= Average protein deposition/ average protein intake.

Lysozyme activity
Blood samples (1mL) were obtained from 3 randomly selected fish per tank at the end of the growth trial. Blood was drawn from the caudal vasculature using heparinized syringes. The blood plasma was assayed for lysozyme activity (Hutchinson and Manning 1996). Bled carcasses were frozen for later proximate analyses.

Digestive proteolytic enzyme activity
Three additional fish per tank were used for the proteolytic enzyme activity assays on whole intestines at the end of the growth trial. The fish were anaesthetized by adding tricaine methanesulfonate at a rate of 30 mg/L and then killed by cervical separation, about 24h after the last meal. The guts were removed by dissection, placed on ice, and uncoiled. The stomachs were excised. The whole intestine was then frozen in liquid nitrogen. The entire procedure for each fish was completed in about 3 min and the samples were stored at −80°C until analyzed. Frozen intestine were homogenized by sonification for 1 min, and centrifuged at 9,400×*g* for 2 min at 4°C. Following centrifugation, the homogenate was collected and stored in small aliquots (100–200 µl) at −80°C until just before use in colorimetric assays of proteolytic enzyme activities. All pH values listed for buffers were measured at room temperature (22°C), and all reactions were run at saturating substrate concentrations as determined for the proteolytic enzyme at 4°C. Blanks consisting of substrate only and homogenate only (in buffer) were conducted simultaneously to account for endogenous substrate and/or product in the tissue homogenates and substrate solutions (German et al. 2009).

Total proteolytic activity
Total protease activity was quantified by detection of primary amines resulting from proteolysis. Succinylated casein was used as a substrate for protein hydrolysis by all proteases within the intestine and compared to trypsin standards. Hydrolysis of the casein substrate results in the release of peptide fragments with free amino-terminal groups. These peptides react with trinitrobenzene sulfonic acid, which forms a colorimetric reaction and formation of yellow trinitrobenzene-peptide products. The color

intensity was measured at 540 nm and was directly proportional to the enzyme activity of proteases in the sample (Bubnis and Ofner 1992; Hatakeyama et al. 1992).

Proximate and fatty acid analyses of the whole body

The body composition of 5 individual fish was analyzed initially before starting the growth trial in outdoor tanks, and after the trial 3 fish per tank (12 per diet) were reserved from the health assays for proximate and fatty acid analysis. The reserved fish were finely ground to get one homogenous sample per tank and subjected to the same procedures described in the digestibility trial, except that fiber analysis was not performed on fish.

Statistical analysis of the digestibility trial data

The mean data per replicate for digestibility, body composition, weight gain (growth rate), survival, PDC, FCR, ANPU, lysozyme activity, and proteolytic enzyme activity will be analyzed by one way Analysis of Variance (ANOVA) with StatView (SAS Institute Inc., Cary, North Carolina) to test for differences among experimental groups. When the differences among treatment means are significant ($P \leq 0.05$ for the indoor digestibility trial; $P<0.01$ for the outdoor feeding trial), Fisher's least significant difference test was used to identify specific treatment differences. Water quality data from the feeding trial was analyzed using repeated measures ANOVA.

RESULTS

Objective 3

Digestibility trial

The apparent protein digestibility (APD, mean±SE, %) of the SOY diet (86.35±0.87) was higher than all others. The APD of the 15 MOR (84.69±0.59) and 30 MOR (83.34±1.10) diets were similar to each other and higher than the APD of the 15 LEU (78.49±0.91) and 30 LEU (74.70±0.52) diets. The APD of 30 LEU was also lower than that of the 15 LEU diet. Overall, leaf meals reduced protein digestibility compared to soybean meal, but diets containing *Moringa* were digested better than those containing *Leucaena*. There was no difference in the apparent lipid digestibility (%) of the SOY (95.83±0.34) and 15 MOR (95.19±0.62) diets. Lipid digestibility of the SOY diet was higher than that of 30 MOR (92.99±1.17), 15 LEU (94.46±0.06), and 30 LEU (92.98±0.24) diets. Lipid digestibility of the 30 MOR and 30 LEU diets was lower than that of the other diets. Both protein and lipid digestibility were inversely related to concentration of dietary fiber.

Objective 4

Feeding trial

Tank 38 was excluded from analysis of growth performance data because we discovered that the tank was not stocked with 100 fish originally, so fish density was lower and fish in that tank were much larger than other fish fed that diet.

Mean individual weight gain ranged from 30.4-34.7 grams, feed conversion ratio ranged from 1.6-1.9, survival ranged from 91.8-97.3%, and there were no differences among diets. Lysozyme activity ranged from 13.1-14.8 units/25 µl plasma and there were no differences among diets. Total proteolytic enzyme activity ranged from 38.8-47.5 µmol/g tissue and there were no diet effects. However, fish size had a significant effect on enzyme activity, and just by chance larger fish were randomly selected from diet 1 replicates for enzymatic analysis. There was a tendency toward decreasing enzyme activity with

increasing fish size, but further analysis is required to interpret this result. Proximate and fatty analysis of whole fish from the feeding trial is still in progress.

DISCUSSION

Objectives 3 & 4

The most likely explanation for reduced nutrient digestibility of the leaf meals compared with soybean meal is the higher fiber content of the leaf meals. Fiber is indigestible to monogastric animals such as fish and can reduce the overall energy and essential nutrients available from the diet (NRC 2011). Despite differences in nutrient availability among diets, fish growth, feed conversion, survival, lysozyme activity, and proteolytic enzyme activity were similar among treatments in the feeding trial. It is likely that the tilapia compensated for any nutrient deficiencies by consuming algae and other natural foods in the outdoor tanks. It is worthy of note that the tilapia were able to make up for diet differences even at a young age, and young fish are usually more sensitive to diet differences than larger fish. The results look promising for increasing use of *Moringa* and *Leucaena* in tilapia diets to reduce diet cost and improve profitability, but the diets need to be tested in a longer study where fish are grown to market size. In addition, economic analysis is needed to help identify the most cost-effective diets for tilapia production.

REFERENCES

Afuang, W., P. Siddhuranju, and K. Becker. 2003. Comparative nutritional evaluation of raw, methanol extract residues and methanol extract of *Moringa* (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile Tilapia (*Oreochromis niloticus* L.). Aquaculture Research 34:1147-1159.

AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis. Arlington, Virginia.

Bubnis, W. A., and C. M. Ofner, III. 1992. The determination of e-amino groups in soluble and poorly soluble proteinaceous materials by a spectrophotometric method using trinitrobenzenesulfonic acid. Analytical Biochemistry 207:129-133.

Bureau, D. P., A. M. Harris, and C. Y. Cho. 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161:27-43.

Creighton, W. 2001. *Moringa oleifera* seed production in Tanzania. In: Anonymous Proceedings of the workshop on development potential for *Moringa* products held in Dar es Salaam, Tanzania from 29 October- 2nd November 2001, pp. [Online] available at http://www.moringanews.org/actes/optima_en.doc Accessed: 1/19/2011.

D'Mello, F. J. P, and D. Thomas. 1977. Animal feed. Pages 30-32 *in* F. R. Rushkin, editor. *Leucaena*: promising forage and tree crops for the tropics. National Academy of Sciences, Washington. DC.

D'Mello, F. J. P. 1991. Toxic amino acids. Pages 22-48 in F. J. P. D'Mello., C. M. Duffus and J. H. Duffus, editors. Toxic substances in crop plants. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge.

De Silva, S. S., and T. A. Anderson. 1998. Fish nutrition in aquaculture. Chapman and Hall, London.

El-Hassan, S. M., A. Lahlou, C. J. Kessi., R. J. Newbold, and C. J. Wallace. 2000. Chemical composition and degradation characteristics of forage of some African multipurpose trees. Animal Feed Science and Technology 86:27-37.

El-Saidy, D. M. S. D. and M. M. A. Gaber. 2002. Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Journal of the World Aquaculture Society 33:297-306.

El-Saidy, D. M. S. D. and M. M. A. Gaber. 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research 34:1119-1127.

Emerson, J. J., R. C. Russo, R. E. Lund, and R. V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada 32:2379-2383.

Eusebio, P. S., and R. M. Coloso. 2000. Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer*. Journal of Applied Ichthyology 16:56-60.

Eusebio, P. S., R.M. Coloso, and R. E. P. Mamauag. 2004. Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides* (Hamilton). Aquaculture Research 35:1261-1269.

Fernandes, A. E., M. A. Gomes, R. Henriques, and J. Combra. 1999. Replacement of fish meal by plant proteins in the diet of Nile tilapia: digestibility and growth performance. Aquaculture International 7:57-67.

Ferraris, R. P., M. R. Catacutan, R. L. Mabelin, and A. P. Jazul. 1986. Digestibility in milkfish, *Chanos chanos* (Forsskal): effects of protein source, fish size and salinity. Aquaculture 59:93-105.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497-509.

Food and Agriculture Organization of the United Nations (FAO). 2006. National Aquaculture Sector Overview. United Republic of Tanzania Fact Sheets. Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO). 2007. Fishstat Plus: Universal software for fishery statistical time series. Version 2.3. 2000. Rome, Italy: FAO Fisheries Department, Fishery Information, Data and Statistics Unit.

Francis G., H. P. S. Makkar, and K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199: 197-227.

German, D. P, B. C. Nagle, J. M. Villeda, A. M. Ruiz., A. W. Thomson, S. Contreras-Balderas, and H. Evans. 2009. Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effects on gut size and digestive physiology. Physiological and Biochemical Zoology 83:1-18.

Hassan, M. R., D. J. Macintosh, and K. Jauncey. 1997. Evaluation of some plant ingredients as dietary protein sources for common carp (*Cyprinus carpio* L.) fry. Aquaculture 151:55-70.

Hassan, M. R., P. K. Roy, and A. M. Akand. 1994. Evaluation of *Leucaena* leaf meal as a dietary protein source for Indian major carp (*Labeo rohita*) fingerlings. Aquaculture 124:65-66.

Hatakeyama, T., H. Kohzaki, and N. Yamasaki. 1992. A micro-assay for proteases using succinylcasein as a substrate. Analytical Biochemistry 204: 181-184.

Hepher, B. 1988. Nutrition of pond fishes. Cambridge University Press, United Kingdom.

Hutchinson, T. H., and M. J. Manning. 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab (*Limanda limanda* L.) sampled from Lyme Bay, U.K. Fish and Shellfish Immunology 6:473–482.

Kale, A. U. 1987. Nutritive value of *Leucaena leucocephala* (subabul). Doctoral dissertation, University of Bombay, Bombay, India.

Kaushik, S. J., D. Coves, G. Dutto, and D. Blanc. 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European sea bass, *Dicentrarchus labrax*. Aquaculture 230:391–404.

Kimbi, E. F. C. 1997. The effects of substituting *Leucaena leucocephala* forage for cotton seed cake as protein supplement for urea treated maize Stover on performance of goat weaner. Master's of Agriculture Thesis, Sokoine University of Agriculture Morogoro, Tanzania, Africa.

Kimoro, B. N. 2003. Potential of selected tropical multipurpose trees as protein supplements for growing goats fed maize Stover basal diet. Master's of Agriculture Thesis, Sokoine University of Agriculture, Morogoro, Tanzania, Africa.

Lloyd, S. W., and C. S. Tucker. 1988. Comparison of three solvent systems for extraction of chlorophyll a from fish pond phytoplankton communities. Journal of the World Aquaculture Society 19:36–40.

Molnar, J. J., A. Rubagumya, and V. Adjavon. 1991. Sustainability of aquaculture as a farm enterprise in Rwanda. Journal of Applied Aquaculture 1:37–62.

Moon, H. Y., and D. M Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus.* Aquaculture 95:97-106.

Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5:600-608.

Norton, B. W. 1994. Browse legumes as supplements. Pages 245–257 *in* R. C. Gutteridge and H. M. Shelton, editors. Forage tree legumes in tropical Agriculture. CAB International, Wallingford, UK.

NRC (National Research Council). 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington, D.C.

Palada, M. C., and L. C. Chang. 2003. Suggested cultural practices for *Moringa*. Asian Vegetable Research and Development Center, Publication no. 03-545, Shanhua Taiwan.

Pantastico, J. B., and J. P. Baldia. 1980. Ipil-ipil leaf meal as supplemental feed for *T. nilotica* in cages. Fisheries Research Journal of the Philippines 5:63-68.

Popma T.*, and M.* Masser. 1999. Tilapia life history and biology. *Southern Regional Aquaculture Center (*SRAC), P*ublication no. 283, Mississippi State University, Stoneville.*

Refstie, S., T. Storebakken, and A. J. Roem. 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162:301–312.

Richter, N., B. Siddhuraju, and K. Becker. 2003. Evaluation of nutritional quality of *Moringa* (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 217:599-611.

Santiago, C. B., and R. T. Lovell. 1988. Amino acid requirements for growth of Nile tilapia. Journal of Nutrition 118:1540-1546.

Sugiura, S. H., 2000. Digestibility. Pages 209-218 *in* R.R. Stickney, editor. Encyclopedia of aquaculture. John Wiley and Sons, Inc., New York.

Spannhof, L., and H. Plantikow. 1983. Studies on carbohydrate digestion in rainbow trout. Aquaculture 30:95-108.

Spinelli, J., C. R. Houle, and J. C. Wekell. 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30:71-83.

Suresh, V. 2003. Tilapias. Pages 321-345 *in* J. S. Lucas and P. C. Southgate, editors. Aquaculture: farming of aquatic animals and plants, Blackwell Publishing, Oxford. United Kingdom.

Tacon, A. G. J. 1985. Nutritional fish pathology. Food and Agriculture Organization of the United Nations. Rome, Italy.

Tacon, A. G. J., and A. J. Jackson. 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. Pages 119-145 *in* C. B. Cowey, A. M. Mackie and J. G. Bell, editors. Nutrition and feeding in fish. Academic Press, London.

Ter' Meulen, U., S. Struck, E. Schulke, and E. A. El-Harith. 1979. A review on the nutritive value and toxic aspects of *Leucaena leucocephala*. Tropical Animal Production 4:113-26.

Thompson, K. R., L. A. Muzinic, L. S. Engler, and C. D. Webster. 2005. Evaluation of practical diets containing different protein levels, with or without fish meal, for juvenile Australian red claw crayfish (*Cherax quadricarinatus*). Aquaculture 244: 241-249.

Wee, K. L., and S. S. Wang. 1987. Nutritive value of *Leucaena* leaf meal in pelleted feed for Nile tilapia. Aquaculture 62:97-108.

Wijkstrom, U. N., and N. J. MacPherson. 1990. A cost benefit analysis of culture based fisheries development in small dams and dugouts. Food and Agriculture Organization of the United Nations Field work paper *1: The Economics of Culture Based Fisheries*, Rome, Italy.

Williams, K. C., C. G. Barlow, L. J. Rodgers, and I. Ruscoe. 2003. Potential of meat meal to replace fish meal in extruded dry diets for barramundi, *Lates calcarifer* (Bloch). I. Growth performance. Aquaculture Research 34: 23–32.

TABLE 1: - The % composition of 10 diets using different ingredients

	Diets								
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**
SFC	84	66	41	21	0	0	0	0	0
SBM	0	0	0	0	0	25	24	20	13
MLM	0	22	41	63	80	0	7	20	39
HM	10	6	12	10	14	69	63	54	42
SFO	3	3	3	3	3	3	3	3	3
MIN.	1	1	1	1	1	1	1	1	1
WM	2	2	2	2	2	2	2	2	2
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

SFC = Sunflower seed cake, SBM= Soybean meal, MLM= Moringa leaf meal, HM= Hominy meal, SFO= Sunflower oil, MIN= Mineral, WM= Wheat meal

TABLE 2. —The major categories of antinutritional factors (Tacon 1985).

Group	Antinutritional factor
Proteins	Protease inhibitors, hemagglutinins.
Glycosides	Goitrogens, cyanogens, saponins, estrogens.
Phenols	Gossypol, tannins.
Miscellaneous	Anti-minerals (e.g. phytic acid), anti-vitamins, anti-enzymes, food allergens, microbial/plant carcinogens, toxic amino acids

TABLE 3. —Composition of the reference diet for a digestibility trial with Nile tilapia. The control diet for the feeding trial was similar but contained 36% protein and wheat midds in place of chromic oxide.

Ingredients	Percentage inclusion
Soybean meal	50.00
Cottonseed meal	7.50
Corn	16.50
Wheat middlings	22.00
Fish oil/soy oil (1:1)	2.00
Vitamin mixture[a]	1.00
Mineral mixture[a]	1.00
Chromium oxide[b]	0.005

[a] Same as Moon and Gatlin (1991).
[b] Chromic oxide will be purchased from Sigma-Aldrich Corp., St. Louis, Missouri.

Impact Assessment of CRSP Activities in the Philippines and Indonesia

Sustainable Feed Technology/Activity/09SFT06NC

Remedios B. Bolivar
Central Luzon State University
Science City of Muñoz, Philippines

Evelyn Grace T. de Jesus-Ayson and Maria Rovilla J. Luhan
Southeast Asian Fisheries Development Centre – Aquaculture Division
Iloilo, Philippines

Hasan Hasanuddin and Coco Kokarkin
Ujung Batee Aquaculture Center
Banda Aceh, Indonesia

Upton Hatch
North Carolina State University
Raleigh, North Carolina, USA

Kevin Fitzsimmons
University of Arizona
Tucson, Arizona, USA

Russell J. Borski
North Carolina State University
Raleigh, North Carolina, USA

ABSTRACT

A series of activities to promote training and to assess the impact of technologies and management practices aimed at improving incomes for small-scale aquaculture farmers in the Philippines and Indonesia was undertaken. In the first phase a workshop consisting with 66 stakeholders was held to provide additional training on reduced feeding practices and least-cost feed formulations and manufacturing technologies that limit the cost of producing tilapia. A survey was also conducted to assess the current production practices of tilapia aquaculture with particular emphasis on evaluating the adoption rate of reduced feeding strategies developed under the CRSP project. Fifty-eight individuals were interviewed and approximately half used reduced feeding strategies, mostly the delayed onset of feeding practice, where feed costs are reduced with little impact on total yield. Based on the survey data, total feed costs were reduced and net return was improved by 46% when the feed reduction strategy was utilized. Based on these results it would appear that tilapia farmers are adopting feed reduction strategies at a high rate and that this results in improved incomes. Considering the size of the tilapia industry, which is the fourth largest in the world, it would appear CRSP research and outreach has positively impacted the livelihoods of an untold number of families.

In a second phase of this investigation we expanded our training on milkfish integrative production systems and feed management practices and on value-added processing of seaweed and milkfish to various islands in the Philippines, including Mindanao, Palawan and Iloilo. Informal interviews and consultation with farmers, operators, investors, and government personnel revealed 3 major constraints to

production – reliable source and consistent supply of good quality fingerlings, prohibitive cost of feeds, and marketing. An additional concern in some locations was the periodic low water quality associated with milkfish cage clusters that result in emergency harvests. To address these concerns CRSP personnel conducted four workshops that provided information and training on how production and economic efficiencies can be improved through some technical interventions. This included ways to improve feeding strategies and management such as the utilization of alternate day feeding strategies shown through recent CRSP research to reduce feed inputs by as much as 30%. Growers are now currently testing this on farm in production marine cages. Another way is to apply the concept of polyculture or integrated multi-trophic aquaculture in the grow-out of milkfish to increase profit and reduce negative impact to the environment. SEAFDEC – CRSP project personnel provided information on integrative culture systems that incorporate sandfish (sea cucumber), seaweed and rabbitfish into milkfish systems. To enhance women's opportunity to improve household welfare through sale of products in local market, a final workshop provided training on seafood safety; marketing techniques; milkfish processing, deboning and marinating; and on seaweed preparation and cookery. Twenty-five women participated in the workshops with the intent on supplementing their household incomes through local market sale of value-added products and for enhancing household nutrition.

The AquaFish CRSP aquaculture program in Aceh, Indonesia has focused on promoting the adoption of best aquaculture practices for seaweed-shrimp/finfish polyculture systems. These systems involve a more sustainable use of small-scale tambak culture systems that incorporate seaweed (*gracellaria)*, shrimp and fish. The third phase of these studies was to assess the impact training activities had on adoption of seaweed-shrimp/finfish polyculture in Aceh, based on interviews, farm visits and data collection. We found that 100 farmers with around 400 ha of farm area have adopted polyculture practices for shrimp and/or finfish growout in various Aceh communities. This is a relatively new culture practice that has recently been adopted. A marketing agreement has been achieved for purchase of seaweeds, which should provide additional income for fish/shrimp farmers that adopt more sustainable polyculture techniques.

Our fourth objective was to develop additional extension podcast modules to promote impacts of AquaFish CRSP research and outreach activities. The podcast approach is far thriftier, more easily updated, and more efficient than the distribution of printed media. With the continued growth of smart phones, MP3 players, computers and other devices in throughout the world we anticipate the Podcast will be a highly attractive extension tool for dissemination of information on aquaculture. To this end three podcasts were produced and uploaded onto iTunesU to demonstrate how alternate-day feeding can reduce costs of milkfish culture in marine cages and brackishwater ponds. Two of these podcasts reflect translations to Tagalog, the primary Filipino language, and Ilonggo, the primary dialect with in the Iloilo region of the Philippines, where milkfish culture is an important form of livelihood. Four other podcasts highlighting the activity of CRSP programs in other countries are currently under development.

INTRODUCTION

A series of activities were completed to promote additional training and to assess the impact of technologies and management practices aimed at improving incomes for small-scale aquaculture farmers in the Philippines and Indonesia. They included work to promote accomplishments from the previous Aquaculture CRSP as well as our current AquaFish CRSP projects.

AquaFish CRSP work in the Philippines and Indonesia was designed to develop and implement strategies that will improve the cost effectiveness, sustainability and income opportunities of farming fish in the Philippines and Indonesia and the subsequent livelihood of their people. Tilapia and milkfish are the two most prominent finfishes cultured in the Philippines and their culture is expanding rapidly both in inland and coastal regions. Feed is the most costly component of fish farming, representing as much as 80% of total production costs for tilapia and 60-70% for milkfish. Therefore, procedures that reduce the amount of feed or its cost without negatively impacting harvest quality or yield can improve farmers' incomes.

Through delayed onset, alternate day, or sub-satiation feeding, or a combination of the three, research has shown that the amount of feed required to culture tilapia in ponds can be reduced by as much as 50% with little or no impact on the total yield of harvested fish. These practical strategies to improve production efficiency of tilapia can increase the incomes of farmers. Anecdotal information has suggested some farmers are adopting these new feeding strategies in the Philippines, including those who conducted the original research trials on their farms, but the extent to which this has occurred was uncertain, prior to this assessment. This may arise from the lack of farmers' knowledge of alternate feeding strategies. Alternatively, farmers may be utilizing new methods, but this had not been quantified. Therefore, one of the aims of this investigation was to conduct additional training on alternative feeding practices to farmers and to survey households to assess the extent to which new feeding practices are being adopted by the tilapia farming communities. These are critical elements to promoting new technologies developed by CRSP as well as assessing the impacts of the technologies.

Milkfish culture is the largest finfish aquaculture industry in the Philippines. As part of the Philippine government's food security and poverty alleviation programs, expansion of milkfish culture is a high priority, both to wean fishers off capture fisheries and to increase income of farmers and fishers alike, whose poverty levels are disproportionately high. Much of the growth in milkfish production is in cage culture in marine or brackishwater coastal areas. Cage culture of milkfish is done at higher densities and with significantly greater inputs of artificial feeds. This practice, however, has led to wastage of artificial feeds and to excessive nutrient loading in receiving waters, exacerbating pollution problems and contributing to periodic fish kills in areas of intensive milkfish culture. It also appears many farmers use substantially more feeds than recommended. Our research results suggest that a reduction of 2-3% below the recommended daily feed ration during the early fingerling stage can produce fish without substantially reduced yields. Moreover, feeding on alternate days rather than the typical daily practice can produce a substantial cost savings in feeds by as much as 50% with limited impact on yield. We have also recently evaluated an integrative milkfish culture system that utilizes co-culture of seaweeds, sea cucumber, and/or rabbitfish with milkfish. The integrative, polyculture system has potential to reduce nutrient overload seen around milkfish culture clusters while simultaneously providing additional income opportunities to farmers through seaweed, sea cucumber and rabbitfish markets. In this investigation we expanded training on alternative feeding practices and on integrative culture systems for milkfish from one location in Guimaras to additional islands where milkfish culture is gaining popularity or is even more prominent, namely in the Mindanao as well as other areas within Visayas regions of the Philippines. Expansion of workshops to these regions has provided wider outreach and training to farmers and communities while enhancing capacity building through the participation of additional local and regional government agencies and other stakeholders in the Philippines.

In Indonesia and the Philippines, interest has heightened in diversifying aquaculture crops, following the realization that intensive shrimp farming practices contributed to the deterioration of water quality in the mangrove coastal habitat. In our recently completed AquaFish CRSP project, we have conducted a series of workshops on incorporating seaweed in the polyculture of shrimp and fish as a more sustainable and environmentally benign, and profitable form of aquaculture. Although some farmers have adopted these polyculture practices, the number of farmers, hectareage, and communities impacted had not been assessed. Therefore, we conducted farmer surveys in Aceh, Indonesia. The project collaborated with the Host Country Institute, Ujung Batee Aquaculture Center, the primary research and extension center in the Aceh province of Indonesia, to collate data and provide a full assessment of the impact of CRSP-funded training programs on alternative and sustainable management practices for shrimp and finfish farming communities of Aceh.

Podcasting is a wide-open, attractive format for digitizing procedures, technologies, pictures, data, and video clips set to music that can accommodate practically any digitized material that is desired. Unlike traditional brochures or fact sheets, it can be updated quickly and easily, and the updates are

distributed automatically; unlike journals, texts, and fact sheets, they do not become obsolete. New advances can be incorporated into updates of old presentations for automatic distribution to holders of free subscriptions. Podcasts are an excellent means to convey information to the public, to include farmers, agribusinesses, extension personnel, congress, USAID and other stakeholders. We have produced of a series of short podcasts to introduce aquaculture technology and other information focused on best management practices and feed reduction strategies for improving the efficiency of Nile tilapia culture, developed primarily in the Philippines. An integral part of these podcasts has been the presentation of specific impacts that target indicators for the new Developmental Themes Advisory Panel reporting requirements of USAID. As a final component of this investigation, we expanded development of podcasts modules beyond those related solely to extending information on cost-effective tilapia culture technologies and best management practices, to conveying information on feeding practices that reduce milkfish production costs and sustainability. We are also in the process of completing several other podcasts that highlight some successes of research at other AquaFish CRSP sites in Southeast Asia, Latin America and Africa.

Collectively, this investigation has addressed two primary goals: 1) the promotion of technologies to improve incomes and livelihood of farmers and fishers, and 2) the assessment of the impact of research conducted under the AquaFish CRSP and previous CRSP programs.

OBJECTIVES

1. Provide training for and assess the impact of technologies and management practices that can improve incomes for small-scale aquaculture farmers in the Philippines and Indonesia.
2. Conduct extension activities and assess the impact of feed reduction strategies on tilapia culture in Central Luzon, Philippines
3. Enhance capacity building, provide training and assess impacts of alternative feeding practices, integrative culture systems, and value-added processing for milkfish production across a broader range of the Philippines.
4. Assess impacts of seaweed polyculture on fish and shrimp farming in Aceh, Indonesia
5. Produce podcast modules to promote outreach and impacts of AquaFish CRSP research in the Philippines, Indonesia and other host country sites in Southeast Asia, Latin American and Africa.

1. Conduct Extension Activities and Assess the Impact of Feed Reduction Strategies on Tilapia Culture in Central Luzon, Philippines

Additional training on alternative feeding practices and least-cost feed formulations and manufacturing technologies that reduce the costs of producing tilapia was provided to farmers, feed manufacturers and extension personnel. A survey of tilapia farming households was also done to assess the extent to which new feeding practices are being adopted by the tilapia farming communities. These are critical elements to promoting new technologies developed by CRSP as well as assessing the impacts of the technologies.

Extension Activities: A workshop on "Tilapia Feeding Strategies and Feed Manufacturing: Meeting Global Challenges" was conducted at the Philippine Carabao Center, adjacent to the Central Luzon State University campus in Nueva Ecija, Philippines in August 2011. This area lies within the primary tilapia aquaculture area of Central Luzon, and CLSU is central to disseminating new technologies to the tilapia industry. 66 people attended the workshop. This included farmers, representatives of the 5 primary tilapia feed manufacturing companies, and government (Bureau of Fisheries and Aquatic Resources) and university research and extension personnel who are key in informing the industry on new culture practices. A series of presentations and discussions on the cost benefits of delayed onset, alternate day,

or sub-satiation feeding was provided. This included presentations by farmers who participated in the on-farm trials that originally demonstrated the improved cost-effectiveness of utilizing delayed and/or reduced feeding strategies over the standard, daily feeding protocol in pond growout of tilapia. Other presentations provided information on hatchery best management practices and the value of utilizing lower cost fishmeal free and low protein diets in growout of tilapia. Various Podcast modules showing the cost-benefit analyses of using delayed or reduced feeding protocols were also shown. This workshop complemented an additional one previously conducted in the Pampangas region of Central Luzon (see investigation 09SFT04NC) where information on cost-effective feeding strategies and diets was also disseminated to farmers and others in the tilapia supply chain.

IMPACT ASSESSMENT

METHODS

A survey was conducted in 2011 to assess the current production practices of tilapia aquaculture with particular emphasis on the feeding strategies involved in the AquaFish CRSP project. The project focused on research at the Freshwater Aquaculture Center (FAC) at Central Luzon State University (CLSU) led by Dr. Remedios Bolivar of CLSU in collaboration with Dr. Russell Borski of North Carolina State University (NCSU). The project's central research goal hypothesis was investigation of the feasibility of reduced feeding as an improved management strategy that reduces costs of tilapia culture and can improve incomes of farmers. The results of the research were disseminated to private and public aquaculture sectors through CLSU training sessions, presentations and individual interactions.

The survey was led by Drs. Remedios Bolivar of CLSU and Upton Hatch of NCSU and conducted by CLSU and NCSU interviewers using a structured interview methodology with tilapia managers and owners in the major tilapia growing region centered in the Pampangas area. Data from 58 completed responses were compiled into a database and a summary of these results is presented in Table 1. The selection of survey participants was largely based on past interaction with CLSU extension personnel, activities and training workshops. The database and discussion of these results are organized into sections on respondent socio-demographics, farm characteristics, and management practices. The number of survey observations was adequate to provide an assessment of current production practices, and evolving use of reduced feeding strategies, of the typical Philippine tilapia producer in the dominant growing region. The supply chain analysis of Drs. Wilfred Jamandre of CLSU and Upton Hatch of NCSU, that was a separate investigation of this project, clearly demonstrated the dynamic growth and evolving nature of tilapia production in the Philippines. Philippine tilapia producers pursue a growing array of production and marketing strategies to meet this growing demand.

RESULTS AND DISCUSSION

Socio-demographics (Table 1). The region covered by the survey was concentrated in Pampangas but also included a few respondents from Bulucan and Nueva Ecija; The respondents included 33 owner-operators, 23 caretakers, one family member and one hired professional manager; 47 were male and 11 were female. Average age of owners was 47 with a range of 28 to 71; age of operators and caretakers was 43 with a range of 18 to 72. The typical tenure status was traditional ownership (40), but leasing was also common (14) and there were 4 respondents who managed ponds under both of these arrangements. Share tenant status (kasama) was thought to be a possibility, and the project team prior to conducting the survey interviews expected such respondents, but none were present in the final completed data set. Lease cost in Philippine pesos (Php/ha/crop) ranged from 7,000 to 20,000; experience of survey respondents with tilapia farming averaged 10 years and ranged from 1 to 25. Eight respondents used a *P. vannamei* polyculture and one used catfish.

Farm Characteristics (Table 1). Pond area owned or managed by the respondents averaged about 5 ponds totaling 17 ha and most ponds (approx 80%) were between 1 and 10 ha; the entire farm was approximately 21 ha. Three were under 1 ha - the smallest farm was only one pond of 0.3 ha. There were 9 respondents with 10 or more ha; the farm with the greatest area was 282 ha and the most ponds were 50. In summary, the socio-demographic profile of the typical respondent was a male in his 40's who lived in the Pampangas region and owned and operated a 20 ha farm that had 5 ponds averaging approximately 3 ha each.

Water source was an irrigation canal (31) or river (20), pond depth averaged 1.7 m, and soil type was either clay (45) or clay-loam (13). Salinity during low tide was 0, but at high tide there were 22 farms that had non-zero pH measurements that were typically in the 3-8 pH range. The ponds with these higher high tide salinity measurements often used polyculture of P. *vannamei* and tended to stock *T. mossambica.*

Management Practices (Table 1). The FaST strain of tilapia dominated (41) and BFAR-GetEXCel (11) and GIFT (6) were also used; most purchased their fingerlings from private sources (53) and only 5 depended solely on raising and stocking their own fingerlings. Density averaged about 50,000 per ha, size was typically 22 (grading size); average price per fingerling was 0.39 PhP and ranged from 0.34 to 0.50.

The average harvest yield was 5,000 kg/ha comprised of 200 g fish and accomplished in a 5-month culture period. The size at harvest ranged from 4 to 7 per kg and averaged 4.9 per kilogram or about 205 g. According to the supply chain analysis of Wilfred Jamandre and Upton Hatch, this size range targets local live markets (150-200g) and only the largest (250g and above) have potential to be marketed through higher volume urban grocery market outlets. The analysis found that fish destined for the latter market were often culled and re-stocked into specialized finishing ponds at lower stocking densities to increase average size demanded by this rapidly growing market opportunity. The lower ranges in the culture period and average sizes obtained from assessment survey respondents may indicate this culling strategy. A large array of production and marketing constraints were mentioned by respondents. Climate related fish kills (26) and typhoons (8) were the most common mentioned. Ten suggested predation from turtles was their most important source of yield loss. Also mentioned were: fish disease from fungi, water quality, availability, cost and quality of fingerlings, feed and market and finance issues.

Almost half the farmers (28 of 58) had adopted the CRSP-CLSU reduced feeding strategy. Respondents typical feeding schedule was completed in 5 months with a range of 4 to 6 months. Of those farmers using reduced feeding, the majority employed the delayed feeding strategy; the most common delay was a week and the average was about 2 weeks; there was one farmer who delayed feeding for a month and two who used a 45 day delay. Reduced daily feeding with an alternate day strategy and percent daily reductions of 50% and 67% were additional possibilities included in CRSP-CLSU research, training sessions and the assessment survey; three survey respondents used these strategies. One used 50% feeding for 2 months and another for 3 months; another respondent used alternate day for 2 months; no respondents used 67% reduced daily feeding. In summary, about half the farmers were willing to try the CRSP-CLSU reduced feeding regimens and the most common was 2-week delayed feeding.

Monthly feeding was accomplished with a staged approach using fry mash, pre-starter, starter, grower and finisher feed formulations. Typically each stage lasted about one month. In the first month all farmers used fry mash (13kg/ha), but depending on growth results, feed availability and marketing strategy, many farmers followed different patterns of formulation use. In month 2, only about 20% used pre-starter; most skipped pre-starter and used about 35 kg/ha of starter. In month 3, most farmers moved to grower formulation with a 62 kg/ha average feeding amount. By month 4, almost all farmers (85%) were feeding grower, averaging 85 kg/ha. Most farmers (72%)) harvested and marketed the crop in month 5, using an

average of 70 kg/ha with an even split between grower and finisher. The farmers that waited until the 6th month to harvest their ponds may have faced an array of impediments. In the previous section, the constraints that farmers mentioned to interviewers were typically related to climate, predation, and financial/marketing problems. The total cost of feed (210,116 Php/ha/crop) was predominantly for grower (54%) and finisher (21%).

Over ¾ of the farmers (79%) used fertilizer and of those 90% purchased inorganic fertilizers with an average total cost of 1,435 PhP/ha/crop. The most common formulation was 16-20-0 used by 31 farmers at an average rate about 1.55 bags per ha and a price of 950 PhP/bag. The second most used fertilizer was 46-0-0 by 18 farmers with an average rate of 1.02 bags/ha and a cost of 1,032 PhP/bag. A small number of respondents also used 21-0-0 or 14-14-1.4.

As discussed above, 48 respondents hired labor and the average cost was 2,770 PhP/ha/crop.

Thirty six farmers reported using chemicals; the most common was lime. Also used were: zeolite, sodium, dolomite, probiotics, and deocare. Total average chemical cost was 1,358 PhP/ha/crop.

Table 1. CRSP Assessment Survey Descriptive Statistics: Philippines. Tilapia. 2011.				
Item	Range		Average	Number
	Min	Max		
Owner-Operator				
Owner				
Age (yrs)	28	72	47	
Gender				47 Male 11 Female
Operator				
Age (yrs)	18	72	43	
Gender				53 Male 5 Female
Experience (yrs)	1	25	10	
Water				
Pond				
Area (ha)	0.3	282	17	
Size (ha)	0.1	10.4	2.8	
Water Source				
Well				6
Irrigation Canal				28
Well + Canal				3
River				20
Harvest				
Weight (kg/ha)	1,857	8,800	5,195	
Avg size (#/kg)	4	7	4.9	
Price (PhP/kg)	49	70.5	61.4	
Culture period (mo)	4	6	5.1	
Stocking				
Species Strain				
GIFT				6
BFAR-GetEXCel				11
FaST				41
Fingerling				
Source				
Own				5
Private				53
Size (Sorting Screen)				
22				49
20				6
17				3
Density (000/ha)	35	71	49	
Cost (PP/kg)	0.35	0.5	0.40	
Feed				
Method				
Daily full feeding				31
50% daily				
Months 1 & 2				1
Month 3				1
67% daily				0
Alternate day				
Last 2 months				1
Delayed				

Table 1. CRSP Assessment Survey Descriptive Statistics: Philippines. Tilapia. 2011.				
Item	Range		Average	Number
	Min	Max		
7				11
14				7
21				4
30				1
45				2
Schedule				
Month 1				
Type[1]	1	1	1	
Amount (kg/ha)	1.0	40.0	13.0	
Month 2				
Type	1	4	2.6	
Amount	4.5	120.0	34.4	
Month 3				
Type	3	4	3.7	
Amount	7.1	150.0	62.0	
Month 4				
Type	3	5	4.1	
Amount	14.3	166.7	84.7	
Month 5				
Type	3	5	4.4	
Amount	14.3	166.7	70.0	
Month 6				
Type	3	5	4.6	
Amount	17.9	138.9	59.9	
Total Feed Cost			210,116	
Fry Mash	429	35,880	8,350 (4%)	
Pre-starter	3,384	21,000	10,595 (5%)	
Starter	4,107	163,567	32,612 (16%)	
Grower	16,311	270,000	114,183 (54%)	
Finisher	12,321	88,889	44,376 (21%)	
[1]1=fry mash; 2=pre-starter; 3=starter; 4= grower; 5=finisher				
Fertilizer				
Use fertilizer				
Y				46
N				12
Type				
Organic				5
Inorganic				40
Both				1
Formulation (NPK)				
16-20-0				31
Amount (bags/ha)	0	6	1.55	
Price (Php/bag)	1,000	890	951	
46-0-0				18
Amount (bags/ha)	0	5	1.02	
Price (Php/bag)	1,070	1,000	1,032	
21-0-0				2
Amount (bags/ha)	0	2.19	1.09	

Table 1. CRSP Assessment Survey Descriptive Statistics: Philippines. Tilapia. 2011.				
Item	Range		Average	Number
	Min	Max		
Price (Php/bag)	780	680	730	
14-14-14				5
Amount (bags/ha)	0	2	.86	
Price (Php/bag)	1,370	1,370	1,370	
Total fertilizer cost (PhP/ha/crop)			1,435	
Labor				
Family				10
Hired				48
Cost (PhP/ha/crop)	0	15,000	2,770	
Chemicals				
Chemical use				
Y				36
N				19
Type				
Zeolite				2
Sodium				9
Dolomite				4
Lime				22
Probiotics				2
Deocare				2
Total chemical cost (PhP/ha/crop)			1,358	

Effects of Reduced Feeding Management Strategy. Table 2 summarizes the comparison of traditional daily full feeding with the CRSP CLSU reduced feeding regimens. Following the survey results discussion above, a focus on the comparison of the farmer management systems used by farmers who adopted reduced feeding with those farmers who continued to use daily full feeding will provide insights into the benefits and costs of the reduced feeding regimen. The effects on production, cost and net returns are all a major focus of the survey and its analysis.

Table 2. CRSP-CLSU Assessment Survey: Production, Cost, and Net Return Estimates. Daily Full Feeding vs. Reduced Feeding. Philippines. Tilapia. 2011.				
Item	Feeding Strategy			
	Daily Full	Reduced	Change	
			Amount	Percent
Production				
Yield (kg/ha/crop)	5,195	5,359	164	3
Average Size (#/kg)	4.9	4.0	0	0
Culture period (months)	5.0	5.1	0.1	2
Total Revenue				
Total Revenue (PhP/ha/crop) (Price = PhP 61)	316,895	326,899	10,004	3
Input Cost (PhP/ha/crop)				
Feed	210,116	183,103	-27,013	-13
Fertilizer	1,435	1,409	-26	-2

Labor	2,770	3,238	462	17
Chemical	1,358	2,054	696	51
Fingerlings	19,460	19,460	0	0
Total Cost				
Total Cost (PhP/ha/crop)	235,139	209,264	-25,875	-11
Net Returns				
Net Return (PhP/ha/crop)	80,435	117,635	37,200	46

The survey had 27 respondents who used reduced feeding and the most common regimen was 2-week delayed feeding, used by 20 farmers. Over 5 MT were produced per hectare per crop for both regimens with reduced feeding resulting in a small increase of 164 kg or a 3% yield increase. Based on a relatively small sample size, these results should be interpreted to indicate no evidence that these yields are different in terms of total weight harvested, which is supported by previous experimental trials. The average size was slightly smaller for reduced feeding.

CRSP-CLSU on farm trials show that reduced daily feeding and alternate day feeding profoundly reduce feed costs with little impact on yield relative to full daily feeding. However, the sample size in this survey was very small for a comparative analysis to the on-farm research trials. Nevertheless, it appears some farmers are adopting the practice.

In light of the evidence above that reduced feeding will not affect harvest weight but could have negative effects on average size, an examination of cost is crucial in assessing the strategies overall economic and financial advantages. These data indicate a 13% reduction in feed cost amounting to over 27,000 PhP savings per hectare per crop, definitely a strong incentive to adopt a delayed feeding strategy. Fertilizer use also somewhat declined by around 2%. Labor showed an increase largely due to the fixed nature of many labor arrangements used by owners who hire labor and managers for their ponds. Chemical costs increased. Stocking densities were not different for the reduced feeding regimens, thus fingerling cost was unchanged. Overall, total cost decreased by 11% with the delayed feeding strategy.

Because some cost are not included, net returns as used in this report might be better viewed as change in net returns, not an absolute estimate of total net returns. That is, the analysis concentrates on aspects of the management regimen that are expected to be different; other costs, e.g. lease debt financing, marketing, are not investigated. Overall, we found that net returns increased by 46% with the delayed feeding providing a net return of almost 37,200 PhP/ha. These results are in line with the expectations of farm trials conducted by the CRSP-CLSU team. Their central hypothesis that reduced feeding could result in lower costs without reducing yield is supported by survey responses. The potential economic effects of smaller sized fish with delayed feeding cannot be assessed within the scope of the survey.

CONCLUSIONS

Table 3 summarizes the performance indicators for the CRSP project in the Philippines, much of which has already been discussed. Participation of local farmers was encouraging and assessment visits, interviews and survey have indicated encouraging production results, shedding light on project accomplishments. Approximately half of the farmers surveyed used reduced feeding strategies and their responses supported previous on-farm production trials that showed the cost of feed can be reduced with little impact on total yield. It appears through these surveys that farmers have adopted reduced feeding strategies at a high rate and that they are also showing a substantial increase in net returns with this management practice. Hence, CRSP research and extension appears to have been effective in improving farm management practices and the potential income of tilapia farmers in the Philippines. Since, the effectiveness of alternate day and subsatiation daily feeding protocols in reducing production costs was demonstrated later than the delayed feeding practice, it is possible that these procedures may be adopted

with greater frequency in the future. Additional and more extensive surveys should be conducted throughout Central Luzon to grasp the full impact of CRSP research on the tilapia community and its estimated 15,000 ha of farms.

Table 3. Assessment Indicators for CRSP-supported Technologies: Reduced Feeding in Philippines. 2007-2011.

Participation	
CRSP supported technology	Survey indicated about ½ of farmers were using reduced feeding.
Interviewed/surveyed	58 survey respondents in Pampangas, Nueva Ecija and Bulucan districts.
Production	
Yield	Reduced feeding did not reduce yield in terms of weight but may have affected size
Area (ha)	Survey indicated about ½ of hectares were managed using reduced feeding.
Household consumption and nutrition	No change
Resources and Cost	
Time	No change
Purchased Inputs	
Fixed	No change
Variable	
Labor (PhP/ha/crop)	+17%
Fertilizer	-2%
Feed (PhP/ha/crop)	-13%
Chemical (PhP/ha/crop)	+51%
Stocking (PhP/ha/crop)	No Change
Returns	
Gross (PhP/ha/crop)	No change
Net (PhP/ha/crop)	+46%
Risk Management	
Biological	No change
Economic	Reduced feeding decreases dependence on most expensive purchased input - feed

2. Enhance Training, Capacity Building and Impacts of Alternative Feeding Practices and Integrative Production Systems for Milkfish Culture in the Philippines.

We expanded our training on integrative production systems and feed management practices for milkfish culture from Guimaras Island (Investigation 09MNE02NC) to Mindanao island where milkfish culture predominates, e.g. Panabo City and Tababuli-Digos, Davao or on Palawan island (Visayas region) and to Palawan island where the industry is beginning to emerge.

The Mariculture Park (MP) was conceptualized by the Philippines Department of Agriculture-Bureau of Fisheries and Aquatic Resources (DA-BFAR) in view of its recognition of the potential of mariculture in increasing aquaculture production and its contribution in meeting targets set in the Comprehensive National Fisheries Development Plan (2006-2025). The objectives of the Mariculture Parks Program include the following: 1) provide employment and an alternative source of livelihood for marginalized and sustenance fisherfolk; 2) develop an area with appropriate infrastructure that will allow fishermen, fish farmers and investors to operate cost-effectively and securely; 3) develop skilled and technically capable fisherfolk to support the mariculture industry; and 4) promote the use of environment-friendly inputs and farm management practices. From the first MP established by BFAR at Samal Island in Davao in 2001, there are as of April 2011, 63 operational mariculture parks, with seven to be launched. BFAR pr
ovides the infrastructure and technical assistance while local government units provide the legal framework and support for the establishment of MPs in their respective areas. Only a few of the MPs established by BFAR has attracted more than 50 locators with about half still being operated by BFAR or local government units (LGUs)as demonstration sites. Nevertheless, production from marine fish cages and fishpens dramatically increased from 4,282 MT in 2001 to over 87,000 MT in 2010 with corresponding increase in production value from P648 million to P8.4 billion. Because of this intensification, it has become imperative to develop more sustainable methods that limit nutrient input in the environment. Indeed, some of these MPs are experiencing poor water quality (low oxygen, high nitrogen, and high sulfides in sediments) and higher risks of crop loss.

Two 5-day survey cum informal workshops on cage culture of milkfish and other species that included interviews with farmers/operators, investors, local government officials and BFAR and Regional Fisheries Training Center (RFTC) personnel was conducted in the Panabu City MP and Tagabuli Bay Park from June 27-July 1 and August 7-13, 2011, respectively. These workshops included the participation of 30 milkfish farmers, operators and technicians. The project examines the technological, environmental, socio-economic and financial components of the operations of the mariculture parks and is outlined in greater detail in the workshop reports submitted. Almost all of the cages are stocked with milkfish. Interviews and consultations revealed 3 major constraints to production – reliable source and consistent supply of good quality fingerlings, prohibitive cost of feeds, and marketing. An additional concern at Tagabuli Bay Park was the periodic low water quality associated with milkfish cage clusters that result in emergency harvests.

To address these concerns SEAFDEC personnel provided information, including that established through AquaFish CRSP research on how production and economic efficiencies can be further improved by some technical interventions. First, is the establishment of multispecies marine fish breeding center and satellite hatcheries, which will consequently produce a stable supply of fry and fingerlings and lower their cost. Second is to improve feeding strategies and management such as the utilization of alternate day feeding strategies shown through recent CRSP research to reduce feed inputs by as much as 30%. To help address the issue on feed costs, RFTC XI Director Andrew Ventura announced that RFTC will set up demo production cages, using alternate day feeding strategies with production runs currently in progress. Additionally, a discussion of the advantages of on-farm feed production using locally-available cheap

inputs was provided. E.G. Ayson informed the group of the study being conducted by R. Bolivar in CLSU in collaboration with R. Borski and his team in NCSU using fermented chicken as protein source. The use of fermented milkfish by-products from processing/value adding activities as replacement for fishmeal is being tried by one enterprising farmer/investor, which is reportedly 30% cheaper than commercial feeds and results in comparable, if not better growth and survival. Third, is to apply the concept of polyculture or Integrated Multi-trophic Aquaculture (IMTA) in the grow-out of milkfish to increase profit and reduce negative impact to the environment. SEAFDEC personnel provided information on integrative culture systems that incorporate sandfish (sea cucumber), seaweed and rabbitfish into milkfish systems.

Two additional workshops on milkfish culture technologies and best management practices to include integrative culture systems and feeding strategies were conducted in Palawan in Peurto Princessa and Narra. We had originally planned to conduct these in Leyte. However, due to the relatively pristine condition of Palawan, the rapid emergence of coastal seaweed, milkfish and marine finfish culture, and the limited training available in the region, BFAR was particularly interested in building capacity and the training in sustainable culture techniques. They BFAR training center helped sponsor the workshops. Twenty-one and 37 individuals participated in these workshops, which were met with considerable enthusiasm. Drs. E.G. Ayson, F. Ayson, M. Luhan and R. Borski and others presented information on gender awareness; milkfish and finfish nursery and growout, feeding strategies, and integrative culture systems.

We also conducted additional workshops on value-added processing of seaweed and milkfish in Leganes, Iloilo (Visayas region) geared to women to enhance their continued participation in aquaculture and opportunity to improve household welfare through sale of products in local markets. Maria Luhan conducted the one-day training workshop that included initial lectures on seafood safety; marketing techniques; milkfish processing, deboning and marinating; and on seaweed preparation and cookery. Recipes were provided. This was followed by skills enhancement exercises in milkfish deboning and practicums on utilizing seaweeds to make crackers, tortillas, salads, and other items that can be marketed locally and/or consumed by households. Twenty-five women participated in the workshops with the intent on supplementing their household incomes with through local market sale of value-added products and for enhancing household nutrition.

3. Assessing Impacts of Polyculture Training on Fish and Shrimp Farming in Aceh, Indonesia

The AquaFish CRSP aquaculture program in Aceh, Indonesia has focused on promoting the adoption of best aquaculture practices for seaweed-shrimp/finfish polyculture systems. These systems involve a more sustainable use of small-scale tambak culture systems that incorporate seaweed (*gracellaria)*, shrimp and fish and that were destroyed in the 2004 tsunami. Based on interviews, farm visits and data collection, this section will provide an assessment of the program's economic impact.

MATERIALS AND METHODS

Visits were made and interviews (20 farmers) conducted during August 2010 and August 2011 at UBAC in Banda Aceh; ACIAR (Australia Centre International Agriculture Research (ACIAR) Samalanga demonstration site in Bireun District; Lancang in Pidie District; Trengadring Multi Species Hatchery in Pidie Jaya District; and Bayu in Aceh Utara District. Negotiations were conducted in Medan and Pidie by Kokarkin, Hasanuddin, Hatch and Fitzsimmons with a buyer and farmers to facilitate a seaweed purchase

agreement in August 2011. These negotiations were successfully concluded and the agreement commenced in October 2011.

The performance criteria used in economic impact assessment will center on the extent to which future incomes of small-scale aquaculture farmers in Indonesia will improve through the seaweed polyculture system recommended by the CRSP UBAC program. Has the project has put in place a system that will facilitate farmer's ability to augment their incomes and family nutrition?
The discussion will proceed with a general description of the existing fish farming system that the polyculture of seaweed will need to complement, followed by some observations on seaweed polyculture. The existing system imposes constraints on the opportunity for seaweed polyculture, but it also provides an opportunity to benefit farmers not only through its own culture but also on positive interaction with the existing system. That is, an evaluation of the economic contribution of seaweed polyculture must be analyzed not solely in the cost and returns to seaweed, but of equal importance the effects on the cost and returns to the other crops in the polyculture system. This contribution can be viewed as a pond management strategy that reduces both biological and economic risk. Benefits of biological risk reduction are largely related to potential disease and water quality improvements and economic risk management is addressed through portfolio diversification that ameliorates potential yield and price fluctuations.

RESULTS AND DISCUSSION

Current aquaculture system. The existing aquaculture system has centered on shrimp, milkfish and tilapia. Cultural practices (BMP's) for these fish and seafood crops and their combinations in various polyculture systems are relatively established. However, optimal stocking and feeding rates have not been extensively researched under local conditions; most of these recommendation and actual farmer practices are based on trial and error and tilapia production in other locations. In addition, optimal feeds, *e.g.* optimal protein level, and product quality have not been studied. Consequently, although farmers are reasonably satisfied with the benefits to aquaculture, they felt they could benefit from more research that documents the best stocking densities, feeding rates and feed quality and also marketing opportunity. fish is an extensively cultured, inexpensive source of fish protein for local markets and its culture has a long consistent history of successful production and marketing. Shrimp culture has experienced severe disease problems with white spot virus; there generally have been few disease problems with milkfish or tilapia. Common shrimp harvest size ranges from 20-40/kg and tilapia are sold at about 400-500g. Typical stocking of tilapia and milkfish is in the range of 2,000-3,000 per ha; feeding at these densities will be 200 kg/ha/crop with a typical price of 6,000 -10,000 rupiah per kg based on feed quality. This feeding rate results in final harvest of about 550kg total (tilapia and milkfish). Seed are often from seed collectors who capture wild stocks.

Tilapia culture is still evolving both in production and marketing sense. Hired labor is generally not used. Neighbors help each other harvest and each farmer does the various tasks during the season on his own. Harvest labor is the only task that requires a substantial effort in a constrained period of time. Sales are almost totally to brokers; however, some farmers rent stalls in local markets with some success. The consumer expects the seller to clean fish as requested. Current 2011 prices per kg are approximately 15,000 rupiah for black tilapia (*nilotica*), 8,000 for red tilapia (*mozambica*) and 15,000 for milkfish. The possibility of cross breeding existing strains with Tilpaia *honorum* has been suggested to increase male population, but not rigorously tested.

Issues and observations. Although initial community demonstration racks for seaweed drying were provided by the AquaFish CRSP, the number of racks has been inadequate. The minimum quantity that the market will purchase is 15 MT as a truckload from a buyer/assembler in Medan. This load provided by the Aceh producers will assist in augmenting product from other sources in the Medan area to reach

the minimum size that the buyer/assembler will need to achieve to sell his product on international markets using a freighter load of 500 MT. For the Aceh farmers to participate successfully in this market supply chain, the capacity of seaweed drying racks is inadequate and expanding this capacity was an important element of buyer arrangement recently concluded.

Seaweed marketing and processing. Although AquaFish CRSP has recently completed additional training on drying and preparation of seaweed, farmers may need more instruction and experience with post-harvest processing and inventory activities. Processing involves weighing, drying, cleaning and packaging and inventory considerations will require storage capacity and time delay to obtain sufficient product to meet minimum buyer load size. These inventory issues will result in a lag before production receipts are received. Polyculture with seaweed will not be an economic success unless this minimum market size can be reached and farmers learn how to prepare seaweed for market efficiently. These processing and inventory requirements are substantially different processes than most fish culture farmers are familiar with.

This forward contracting system for seaweed will depend on establishing trust between farmers and brokers. A forward contracting system will stabilize price for farmers and quantity/quality for brokers; neither the buyer nor seller have any established history or relationship that underlies confidence that both ends of contract will be met. With the help of a trusted government entity, e.g., UBAC, to initiate the process, an evolving market maturation process will lead to eventual functioning of the market without public sector facilitation. Assurance to buyers that the minimum quantity/quality will be available on a consistent basis is crucial on the demand side and price stability is crucial on supply side for Aceh seaweed producers.

Odor. The relationship between seaweed culture and odor in fish harvested is a concern to farmers and consumers that should be investigated more thoroughly. Best management practices need to be established and explained to farmers that minimize odor problem related to seaweed in polyculture ponds. There seems to be some confusion among farmers, extension and researchers, as to the exact context and process that is occurring as it relates to negative odors of fish and shrimp harvested from seaweed polyculture ponds. Several observations were provided that indicate the odor in fish and seafood marketed from polyculture ponds need serious scrutiny. Seaweed start to die within 1-2 months of the culture cycle; at this time, it is not possible to remove affected seaweed due to damage to shrimp/milkfish/tilapia. The odor is mostly a problem in fish – milkfish and tilapia, and particularly milkfish – not shrimp. Several hypotheses are being suggested that should be investigated: that (1) shrimp are able to move under seaweed or mangrove roots to remain unaffected and (2) as long as seaweed coverage in pond area is under 30%, dyeing seaweed and resulting odor in fish, is not likely to occur. These suggestions and others should be investigated to gain farmer's greater confidence in seaweed polyculture.

Shrimp virus. White spot virus in shrimp may be mitigated by polyculture with seaweed and tilapia. This virus, carried by crustaceans, has decimated shrimp culture in the Aceh area. The possibility that these polyculture systems reduce disease risk in shrimp is a major potential benefit to farmers. It is suggested that tilapia in pond canals and polyculture of shrimp with milkfish and seaweed in pond may have excellent disease management benefits. Also, there is evidence that rotation of pond, *i.e.* not growing same species in same pond each crop cycle, is also a potential strategy to reduce white spot damage.

Some farmers overstock in anticipation of losses due to oxygen deficiency related to dying seaweed. Because, in general, shrimp die early in cycle (1-2 months), overstocking may be appropriate because feed will not have been wasted on shrimp that are not eventually harvested. Polyculture with shrimp also mitigates damage of white spot by allowing farmers to continue with fish crops if shrimp are severely

damaged. Although lower shrimp stocking density is yet another potential solution to the white spot virus, these lower densities will likely reduce net returns to the shrimp culture substantially. It might be possible to increase stocking densities of the fish polycultures with the lower shrimp density, but the returns to milkfish and tilapia have been substantially lower than shrimp. The obvious conclusion from this discussion is that more research on BMP's to mitigate white spot is needed.

Current status of seaweed culture. Seaweed polyculture has an excellent opportunity to be incorporated into and provide several important benefits to the existing aquaculture system. However, this potential contribution is only beginning to materialize. Efforts to address several marketing and production constraints have been initiated, but results from these efforts are still pending. Reducing risk and uncertainty inherent in these constraints undercuts attempts to increase adoption and farmer risk attitudes are both expected and addressing them is key to establishing seaweed polyculture as an accepted alternative for aquaculture farmers.

Current production constraints are generally related to seaweed polyculture interaction with other aspects of the polyculture system, e.g., yield, odor, shrimp virus. Support is needed to initiate and continue applied research and extension on (1) relationship between white spot virus and other polyculture (tilapia, milkfish, and seaweed); (2) interaction of odor and crops in polyculture system (tilapia, shrimp, milkfish, and seaweed) and (3) continuous updating of appropriate polyculture BMP's, e.g., species mix, stocking densities, complementarities.
Post-harvest processing and marketing issues are clearly constraining, and are as important as production constraints in establishing Aceh farmer's long-term viable competitive position in the seaweed market supply chain. These are activities that farmers are generally not aware of or well skilled in. The post-harvest chain of events – drying, weighing, cleaning, packaging, storage, selling, and transport – each involve challenges.

Marketing agreement. Although at the end of the CRSP project seaweed had not been marketed, arrangements were in place for a buyer/assembler to purchase one 15 MT truckload of dried, cleaned, packaged seaweed weekly. Approximately 400 ha of seaweed are ready for harvest and at least 20 farmers are prepared to be part of the arrangement. The incentive for the buyer is based on his need to provide a minimum freighter load of 500 MT; this volume requirement has been difficult to obtain from nearby producers in Medan. The buyer will provide the transportation and a small loan to assist in buying materials and paying a labor cost to expand drying rack capacity. The loan will be paid through payments, subtracting 25% of debt from receipts due to farmers. It is anticipated that both of these levels of participation will expand dramatically once profitability is demonstrated.

A farmer representative was designated who will play a key role in the arrangement's potential success; the buyer will deal exclusively with this individual. Sales transactions will be undertaken by the representative. In addition, this representative will be responsible for managing the weighing, drying, cleaning, packaging and storage. The seaweed will be stored in 25 kg bales until the minimum quantity is reached for scheduled deliveries. As success is demonstrated, other seaweed growing areas in Aceh are expected to begin harvesting and additional representatives will be needed in these other locations. Success of this representative will be important in determining whether this marketing arrangement is sustainable and whether other representatives will perform this function as production capacity expands.

The process was initiated in October and is currently still operating at a mutually acceptable level. UBAC plans to provide farmer assistance after the CRSP project termination. If this arrangement is successful, it will provide a mutually beneficial opportunity in assuring that the buyer can meet freighter minimum volume and quality and that the farmers will have an additional income source with a very low input cost.

CONCLUSIONS

Table 4 summarizes the major performance indicators for the CRSP-UBAC program that has been already discussed in detail in earlier sections of this report. Overall, participation of local farmers was encouraging and assessment visits, interviews and survey have indicated encouraging production results, shedding light on project accomplishments and challenges. An estimated 100 farmers and 400 ha of farm area have adopted seaweed polyculture due in large part to the effort of AquaFish CRSP-UBAC program. A marketing agreement has been achieved for purchase of seaweeds, which should provide additional income for fish/shrimp farmers. Household consumption of nutritious seaweeds and the potential to market foods locally has been enhanced through post harvest training in processing, seaweed cookery, and value-added product development. Future work should be directed at optimizing polyculture systems and continued training in processing, inventory, and marketing activities.

Table 4. Assessment Indicators for CRSP-supported Technologies: Reduced Feeding in Philippines. 2007-2011.

Participation	
CRSP supported technology	100 estimated farmers have seeded seaweed in their ponds.
Interviewed/surveyed	20 farmers interviewed in Bireun, Pidie, Jaya and Utara districts.
Production	
Yield	1 MT/ha[1]
Area (ha)	400 ha of seaweed is available for harvest.[1]
Household consumption and nutrition	Small amount may be consumed in household.
Resources and Cost	
Time	High - harvesting, drying, cleaning and packaging
Purchased Inputs	
Fixed	Drying racks/tables – buyer loan
Variable	
Labor (PhP/ha/crop)	Labor cost increased substantially
Fertilizer	0
Feed (PhP/ha/crop)	0
Chemical (PhP/ha/crop)	0
Stocking (PhP/ha/crop)	Transplants from previous crop
Returns	
Gross (PhP/ha/crop)	Additional income from polyculture or fallowed pond
Net (PhP/ha/crop)	Increased cash income – increased labor time and cost
Risk Management	
Biological	Seaweed polyculture can mitigate biological risks associated with tilapia, shrimp and milk fish culture systems
Economic	Portfolio diversification of products from pond reduces vulnerability to price fluctuation

4. Podcasting to Promote Impacts of AquaFish CRSP Research and Outreach Activities

A database of raw materials, pictures, and graphs were assembled for the development of 3 podcasts on milkfish culture. Specifically, the podcasts disseminate work showing the effectiveness of alternate day feeding in reducing feed costs by as much as 30-56% for growout of milkfish in sea cages and brackishwater ponds. One of the podcasts are produced in the English language and two others were modified and translated into Tagalog, the primary Philippines language, and Ilonggo, a primary dialect in the Iloilo, Visayas region of the Philippines. The podcasts are as follows:

1) Alternate-Day Feeding Strategy for Reducing Cost of Milkfish Culture in Brackishwater Ponds and Marine Cages in the Philippines (English)
2) Pag-aalaga ng Bangus sa tubig alat at tabsing sa pamamagitan ng pagbibigay ng pakain batay sa halinhinang araw ng pagpapakain (Tagalog) (English translation: Alternate-Day Feeding Strategy for Reducing Cost of Milkfish Culture in Brackishwater Ponds and Marine Cages in the Philippines)
3) Alternate-Day Feeding Strategy for Reducing Cost of Milkfish Culture in Brackishwater Ponds and Marine Cages in the Philippines (Ilonggo)(Only the verbal language has been translated from English to Ilonggo)

Scripts were all pre-written in an interview style prior to recordings. Original music soundtracks recorded by Dr. Gary Wikfors of the NOAA Biotechnology Branch (Milford, Connecticut), were also incorporated into the podcasts. Podcasts were reviewed by the team, modified where appropriate and then uploaded at the NCSU iTunesU site where downloads, and other data for podcasts will be collected (http://itunes.apple.com/us/itunes-u/milkfish-aquaculture-alternate/id499954016).

We are also in the process of completing several other podcasts that highlight some successes of research at other AquaFish CRSP sites in Southeast Asia, Latin America and Africa.
Due to the tight schedules amongst the US PI and host country PIs it has been difficult to arrange travel for collection of resources for development of the podcasts. Albeit more difficult, this is now being handled electronically. A new student has also been trained in podcasting to replace the one previously involved in production. One podcast is largely written and will be assembled, reviewed and uploaded shortly (Southeast Asia: Determining the Environmental Performance of Marine Shrimp Culture using Life Cycle Assessment). Others (Mexico, Africa, Southeast Asia) are at different stages toward completion, which we anticipate will occur over the next 45 days.

Sustainable Feed and Improved Stocking Densities for Gar (*Atractosteus spp*.) Culture

Sustainable Feed Technology/Experiment/09SFT07UM

Solomon David and James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

Wilfrido M. Contreras-Sanchez
Universidad Juarez Autonoma de Tabasco
Villahermosa, Tabasco, Mexico

Two experiments were proposed for this study. The first experiment investigates multiple treatments of fish-meal substitution (using animal by-products in place of fish meal at 0, 25, 50, 75, 100% substitution) in feed for two species of *Atractosteus* gars, the Cuban gar (*A. tristoechus*) and tropical gar (*A. tropicus*). The experiment using Cuban gars would take place at the University of Michigan (U-M), United States, and the experiment using tropical gars at Universidad Juarez Autonoma de Tabasco (UJAT) in Tabasco, Mexico. The second experiment investigates improved stocking densities of tropical gars and would take place in Tabasco only. Stocking density treatments will be 25, 50, and 100 fish/m^3.

Aquacultured tropical gars were acquired by UJAT and pellet trained for feeding experiments. We are currently awaiting materials for production of the appropriate feed to be used in both Cuban gar and tropical gar experiments. Upon receipt of materials, feeding trials will commence on both species at their respective locations. Improved stocking density experiments will also begin at UJAT upon receipt of funds.

CUBAN GAR EXPERIMENTS

Cuban gars (~13-15 cm) were acquired through multiple shipments during March-June 2011 and were pellet-trained for experimental trials from June-August 2011.

An initial feeding trial using live feed (fathead minnows *Pimephales promelas*) was run for 52 days to establish a baseline for growth rate at 0% fishmeal substitution. The pilot study used 4 replicates each with 3 gars in experimental aquaria. Individuals fed ad libitum on live fish had a mean increase in weight of 395% over the experimental period. Cuban gars (N = 45) are currently prepared for experimental trials at U-M that will begin in 1-2 weeks upon arrival of appropriate experimental feed from UJAT.

A second pilot experiment was carried out to investigate growth rates of Cuban gars on different feed types. Experimental feed consisted of frozen fish (anchovies, used as a proxy for 100% fish meal), freeze dried krill (high-protein non-fish-meal feed), and a high-quality pellet (New Life Spectrum Pellet; primary ingredients were fish meal and krill meal). Initial feed amount was based on 10% of mean fish body weight for each tank. Fish were allowed to feed ad libitum for 30 min, at which point uneaten feed was removed. Amount of feed consumed was also monitored by subtracting weight of remaining feed from amount initially fed. Wet weights and dry weights of each feed type were calculated in order to accurately compare consumption across feed types. Water chemistry (pH, ammonia, nitrite, and nitrate) was also monitored weekly to test for localized effects of feed type on water chemistry. We expected insignificant differences in water chemistry among treatments due to the recirculating aspects of the system. Duration of experiment was 21 days (November 8 – November 29, 2011).

Cuban gars were divided into 3 groups of 3 replicates per group. Group 1 (FISH feed) consisted of 3 replicates, n = 4 fish per replicate; Group 2 (KRILL feed) consisted of 3 replicates, n = 5 fish per replicate; Group 3 (PELLET feed) consisted of 3 replicates, n = 5 fish per replicate. Total N = 42 fish. Individuals were maintained in 170 L fiberglass tanks (1 replicate group per tank) which were connected to a closed recirculating filtration system. Water temperature was maintained at approximately 27 °C using heaters in each tank for the duration of the experiment.

Length and weight were measured 3 times over the course of the experiment (days 1, 14, 21) to determine percent growth and growth rates. Descriptive statistics and ANOVA were used to analyze growth and consumption data. After experiment concluded fish were removed from tanks and placed into a single round 1,000 L tank with high-capacity canister filtration. Fish are currently being fed a combination of pellet and krill feed in preparation for arrival of experimental feed from Mexico (expected in mid-late February 2012).

Mean length did not significantly increase over time in any of the treatments, but this was expected due to the short duration of the experiment. Mean weight increased significantly in fish feed treatment, but not significantly in krill or pellet feed treatments. ANOVA tests indicated that both fish and krill feed treatments experienced significantly higher growth than pellet treatment, however, fish and krill feed treatments were not significantly different from each other. Consumption rates were significantly different among all treatments. Consumption was highest with fish feed (23.97 g/feeding), followed by pellet (15.26 g/feeding), and finally krill (11.48 g/feeding). Krill feed had the highest body mass gained per gram of feed consumed compared to other feed types. As expected, water chemistry did not vary significantly among any treatments likely due high water turnover rate by the recirculating system. Descriptive statistics for the experiment are found in Table C1.

Table C1. Descriptive statistics for second pilot study of Cuban gar growth (weight) for 3 different feed types. All values are in grams unless otherwise noted. Day 1 and Day 21 are mean weight ± 1 standard error. Fish feed type was the only type to experience significant increase in mass compared to starting mass. Final mass of fish and krill feed treatments were significantly greater than pellet feed treatment. Mean consumption was significantly different among all feed types.

Feed Type	Day 1	Range	Std. Dev.	Day 21	Range	Std. Dev.	Percent Growth	Mean Consumption (g/feeding)
Fish	44.60 ± 4.31	30.80-88.20	15.55	59.27 ± 5.50	42.50-109.60	19.04	31.78*	23.97*
Krill	43.93 ± 4.75	20.20-98.20	18.41	54.87 ± 5.43	28.60-114.50	21.01	24.92	11.48*
Pellet	54.53 ± 9.08	18.10-122.40	35.15	59.03 ± 9.30	22.40-128.40	36.02	8.25	15.26*

*indicates significant difference based on ANOVA tests

We believe the fish and krill feed treatments experienced greater growth for several possible reasons. Although all fish were trained to consume all three types of feed prior to the experiment, certain feed types seemed to hold greater appeal to the gars and therefore may have played a role in consumption and therefore growth. Fish feed is the most natural feed type to gars; even though feed was non-live chopped fish, the gars readily accepted this feed type and are naturally able to metabolize the food. Krill feed has a bright color and strong odor, both of which attracted the gars. Krill has also been shown to be highly digestible, and gars of all species have been shown to consume invertebrates such as insects and crustaceans. The pellet feed was not brightly colored, and did not have as strong an odor as krill. Both of these differences may have made the feed less appealing to the gars. Additionally, the pellet feed consisted of additional materials (wheat meal, brewer's yeast, spirulina) which may not have been as

digestible to the gars compared to fish and krill meal, therefore growth may not have been optimal on pellet feed.

These issues will all play a role in the full experiment, and we will adjust accordingly. All feed in the primary experiment will be pellet feed, so "appeal" of feed should not be as large a factor. Digestibility of non-fishmeal components will likely be the largest factor. Given these results, we hypothesize that the feed type with the highest amount of fishmeal will experience the highest consumption and the highest growth.

TROPICAL GAR EXPERIMENTS

Gars are top-level predators in their native ecosystems and are characterized by their elongated jaws, cylindrical bodies, and diamond-shaped ganoid scales. Their maximum size and age varies with species from approximately 80 cm and 10 years (shortnose gar) to 300 cm and over 70 years (alligator gar). Gars are generally polyandrous in reproductive strategy, with multiple male individuals spawning with 1-2 females. Gars spawn during late spring and early summer in temperate regions and during the rainy season in tropical regions. Growth is extremely rapid, with all species capable of reaching 30 cm or more in their first growing season (young–of-the-year alligator gar can reach over 30 cm, 250 g in 3 months).

Gars are excellent candidates for aquaculture as they exhibit rapid growth to large sizes, are highly resistant to disease, can be maintained at high densities, readily adapt to artificial feed at early life stages, and are highly tolerant to low water quality conditions due to their air-breathing abilities (Alfaro et al. 2008). Their tolerance of low water quality via aerial respiration also allows for a less complicated technological system for aquaculture, as opposed to other fishes which may require considerable aeration and water turnover. Gars are therefore well-suited for culture in developing regions.

Much progress has already been made in the aquaculture of *Atractosteus* gars (tropical, Cuban, alligator), primarily in regions of Mexico, Cuba, and the southern United States. Broodstock for all three species have been established and are currently maintained in their native regions, and juveniles have been released to help restock diminishing wild populations. Further efforts are being made in the southern US to protect alligator gar populations and manage them as a viable sport fishery, as well as increase its potential as a food fish. Gars are already popular food fish in various regions of Mexico and Cuba.

Due to their unique appearance and predatory nature, gars are becoming increasingly popular in the ornamental fish trade. Gars have been sought-after aquarium fish in Southeast Asia for many years, and are growing in popularity in the United States and other countries. The Florida gar, native to only a small portion of the southeastern United States, is the most popular aquarium species of gar in the US (usually wild-caught) and most readily available abroad. Prices in the United States range from $15-$40 USD for 20-35 cm individuals. Other gar species at similar sizes command a much higher price largely due to their rarity in the aquarium hobby, such as $200 USD for an individual tropical gar and over $300 USD for a Cuban gar (in the United States). Tropical and Cuban gars are also highly valued overseas; in Singapore 15 cm tropical gars average $150 USD and Cuban gars $ 400 USD. Ironically, tropical and Cuban gars are among the most commonly cultured gar species. Specimens exhibiting genetic mutations in pattern or coloration (i.e. melanistic, xanthochroic, leucistic) command an even higher price, ranging from $1000 to over $5000 USD. Hybrid gars, although rare in the trade, are also much sought-after.

In this study we aim to determine optimal stocking densities for grow-out and the possibility to substitute fishmeal, using by-products.

Experiment 1. Determine Optimal Densities for Rearing Tropical Gars

METHODS AND MATERIALS

This study started using tropical gars averaging 13.00 g in weight and 15.66 cm in length. Fish were randomly allocated in a recirculation system composed of $1m^3$-tanks in three treatments (25, 50 and 100 fish/m^3) run with three replicates. Every 15 days we registered growth in total weight and length. During the experiment we registered temperature, dissolved oxygen and pH. Tanks are cleaned by siphoning the bottom to eliminate wastes from fishes and remaining feed. All fish were fed *ad libitum* three times a day and total food consumed was recorded daily. Mortality was observed daily in every tank.

Results were analyzed using a one way ANOVA to determined if there were significant differences ($p<0.05$) between treatments before, during and after the experiment. The package STATGRAPICHS 5.0® was used for statistical analysis and SIGMA PLOT 11® for graphical representation.

RESULTS

This experiment was conducted using 3-month old tropical gars. Average weight and length were 13.00 g and 15.66 cm, respectively. After 15 days of experimentation, no significant differences were observed between treatments for length or weight (ANOVA; $P>0.05$). At this time, fish stocked at 100 fish/m^3 had a mean weight (SD) of 14.94 g (1.13), with 15.56 g (1.22) for 50 fish/m^3 and 17.42 g (0.98) for 25 fish/m^3. Average length ranged from 15.98 to 16.54 cm. Despite no indication of significant differences, the fish with lower density had a slight tendency for better growth. This pattern was confirmed at 30 days of experimentation when statistical differences between densities were detected (ANOVA; $P<0.05$). Highest growth was detected at 25 fish/m^3 having 27.00 g (± 3.23) in weight (Fig. 1A), and 19.08 cm (± 0.58) in length (Fig. 1B). At this time, differences between the lowest and the highest growth groups averaged seven grams.

After 45 days, differences were larger between treatments with 25 and 100 fish/m^3. Highly significant differences were found between treatments for average weight (ANOVA; $P<0.01$). The treatment with 25 fish reached 36.19 g ± 2.82; while treatments with 50 and 100 fish/m^3 reached 31.47 (2.62) and 22.88 (2.64) respectively (Fig. 2). Significant differences were also found in length, being higher for the 25 fish/m^3 (20.36 cm ± 0.53) while the other two treatments reached 20.05 (0.77) and 18.37 (0.69), respectively.

There were no significant differences in survival between treatments (X^2; $p>0.05$), with survival of 100%, 98.6%, and 98.6% for 25, 50 and 100 fish/m^3, respectively.

DISCUSSION

Stocking density is a very important aspect in fish culture systems because it has a significant impact in growth. In our study, the best results were found in the lowest density (25 fish/m^3) compared to densities of 50 and 100 fish/m^3 after 45 days. Similar results were found by Ramon (2003), who found best growth at densities of 10 and 15 fish/m^3 in comparison with 5 fish/m^3. He also mentioned a tendency to gain more weight when tropical gars were stocked at higher density, however, information was not provided. Rivera and Márquez (2001) evaluated the effect of stocking density on growth and survival in gar larvae in captivity, and found no differences in weight or length between 1 and 40 larvae/L.

Experiment 2. Determine the Degree to Which Fishmeal Can Be Replaced Using By-Products in Gar Feeds

In this case we used fish weighing 14.40 g with an average length of 15.79 cm. Four treatments were evaluated (25, 50, 75 and 100% fishmeal substitution) run in three replicates. The fishes were placed in a recirculation system (every tank stocked with 10 fish). Fish length and weight will be measured every 15 days to determine growth over the course of the experiment. From these growth data we will determine optimal feed types and stocking densities. From these trials we hope to develop low-cost and environmentally friendly methods (such as using lower-fishmeal content feeds) for culture of the tropical gar.

This experiment was recently started. Fish averaged 14.40 g in weight and 15.79 cm in length. No significant differences were detected among treatments at the beginning of the experiment (ANOVA; P> 0.05). Fish will be sampled every 15 days to determine significant differences using a one-way ANOVA.

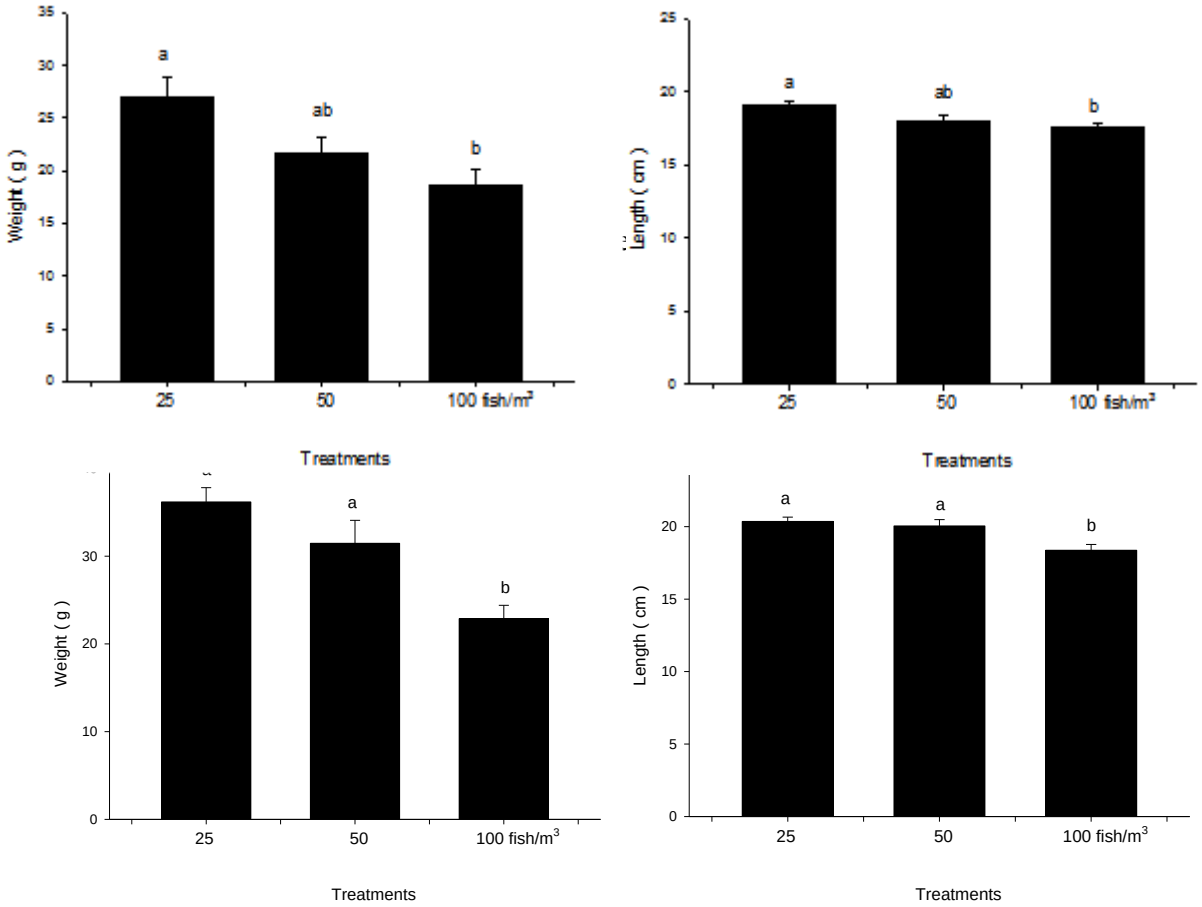


Figure 2. Average weight and length for the three densities evaluated in this study after 45 days of experiment.

SPOTTED GAR EXPERIMENTS

In addition to growth experiments for Cuban gars, we conducted growth experiments comparing two populations ("peripheral" from the Great Lakes region and "core" from the Mississippi River basin) of spotted gars (*Lepisosteus oculatus*) to test for effects of latitudinal and countergradient variation in growth rates between populations (Figure 1). Identifying populations of fishes with higher growth rates at different temperatures can be beneficial in aquaculture by increasing production due to optimal growth. These "strains" of fishes would be the ideal choice for optimizing production (as seen in other species such as salmonids and centrarchids).

Several northern populations of fishes have been shown to have faster growth rates than conspecifics from southern latitudes when reared in common garden environments. We tested for this variation in growth of young of the year (YOY) spotted gars in the following experiments. Based on our findings, other aquacultured lepisosteids, such as the much larger alligator gar (*A. spatula*) or more commonly cultured tropical gar, may exhibit similar interpopulation variation in growth. We propose to expand our research to other gar species and air-breathing fishes as well.

Countergradient variation, or more generally, latitudinal variation, has not been studied in gars; the disjunct distribution and primitive ancestry of the spotted gar makes it a unique model species for investigation of this phenomenon. To explore potential differences in core and peripheral gar populations in the context of countergradient variation theory, we compared growth rates for the first growing season between core and peripheral populations of the spotted gar. Our primary objective was to investigate differences in life history patterns, specifically growth rate in the first growing season, between the Great Lakes (peripheral) and southern United States populations (core) of spotted gars using common garden experiments. Our second objective was to determine whether any potential variation in growth rate might be explained by countergradient variation theory. We hypothesized that spotted gars from the peripheral population would exhibit a faster growth rate and higher capacity for growth at all temperatures than spotted gars from the core population. And further, we hypothesized that this variation in growth rate between populations is evidence of countergradient variation in growth of spotted gars.

METHODS

Spotted gars were acquired from two major sources to represent the core and peripheral populations. Core population representatives were collected via colleagues at Nicholls State University (Thibodaux, LA) in late spring 2009 from several localities in southwestern Louisiana using experimental gill nets, and peripheral population representatives were acquired from several inland lakes in southern Michigan. Fish from Louisiana were the progeny of wild-caught individuals from 2 localities in the Barataria estuary system (Bayou Chevreuil and Golden Ranch) and 1 locality in the Terrebone estuary system (Chacahoula Swamp) collected in March-April 2009. Individuals from the core populations were intermixed in order to reduce potential genetic bias from a single locality, and the same was done for individuals from peripheral populations. Adult fish from all core populations were maintained together in an indoor tank, and spawning was induced at 21°C using Ovaprim™ (Syndel Laboratories) injections at a concentration of 2.0 mL/kg body weight. Ovaprim™ was introduced via intramuscular injection near the anterior base of the dorsal fin, and spawning occurred within 24-48 hrs of injection. Viable embryos from this spawning event were then collected from the tank and approximately 150 specimens were shipped overnight to the University of Michigan.

Adult peripheral population representatives were collected in late spring (May) 2009 from five different inland lake localities in southern Michigan using a boom electrofishing boat. Marble and East Long lakes are part of the St. Joseph River watershed, and Round, Carpenter, and Sugarloaf lakes are part of the Grand River watershed. Adults from peripheral populations were maintained together in an indoor tank similar to that of core population fish. Spawning was similarly induced using Ovaprim™ but was not as successful, therefore several adult fish were stripped of milt and eggs to create embryos (approximately

200 specimens). Core population gars will be referred to as LA fish and peripheral population gars as MI fish from henceforth.

Embryos from both populations were raised in separate 38 L aquaria using aeration and daily 50% water changes to maintain water quality. A 25-watt heater was used to maintain consistent temperature (21-23 °C) during the incubation period as well as post-hatch. Sac-fry and free-swimming larvae were maintained in multiple aquaria separated into core or peripheral populations. Once larvae were zooplanktivorous, they were further separated into 3 aquaria per population to better maintain water quality. Zooplanktivorous larvae were first fed small *Daphnia sp*, and then larger *Artemia* adults. Larvae were fed 2-3 times daily to maintain a constant supply of food. Larvae from both populations were fed small (3.0 cm) fathead minnows *Pimephales promelas* upon converting to piscivory. Larvae were further separated roughly based on size into 3 aquaria per population to reduce cannibalism. To estimate early life growth rates during the period from 1-100 days after hatch (DAH) preceding experiment 1, 30 individuals from each population were randomly selected weekly for measurements of length (0.1 cm) and weight (0.1 g). Mean growth rates (cm•d-1 and g•d-1) were then calculated for each population. Once juvenile gars were regularly feeding on medium-sized (4.5-6.0 cm, size range used in experiments) fathead minnows, individuals were randomly selected from each population and placed into experimental aquariums. All selected individuals were acclimated to experimental aquariums for 4-5 days prior to the start of experiment 1. Excess individuals were maintained in separate aquaria (based on population) as replacements if needed and for experiment 2.

Experiment 1

Twenty 75 L aquaria were used for housing YOY spotted gars from both populations (N = 30 fish from each population). Each aquarium was divided equally into three compartments using thin fiberglass screening, which allowed passage of water, but not other gars or feeder minnows. Each individual compartment housed one gar (3 gars per aquarium, total of 60 gars). Each aquarium also contained an air pump-operated sponge filter to maintain water quality and a 50-watt heater to maintain consistent temperature of 22-24 °C. Temperature range was selected based on mean temperatures experienced during the growing season by both populations (Redmond 1964, Echelle and Riggs 1972, Simon and Wallus 1989, Simon and Tyberghein 1991, personal observation). To further maintain water quality, 50% of the water was changed weekly for each tank, with waste material removed via siphon. Overhead fluorescent lights on electronic timers were used to maintain a consistent 12-hour photoperiod during the experiment. Individual spotted gars were fed fathead minnows ad libitum for the duration of the experiment, 62 days for LA fish and 63 days for MI fish. To accomplish ad libitum feeding, a small group of minnows (approximately 5.0-7.0 g total mass) was consistently maintained in each experimental compartment; consumed minnows were replaced and dead minnows were removed to prevent deterioration of water quality.

Individual gars were removed from compartments to measure length and weight weekly as well as at the beginning and end of the experimental period. Mean length and weight were used to determine increase in growth and growth rate (cm•d-1 and g•d-1) over the experimental period. One-way analysis of variance (ANOVA) was used to test for significant differences in initial and end mean length and weight for both populations. Analysis of covariance (ANCOVA), with population and DAH as fixed factors, was used to determine significant differences in growth rates between populations, if any. I assumed a linear model for growth during the experimental period of development for both populations of spotted gars. Increase in length and weight for each population was plotted versus time (DAH or days of experiment) and analyzed using linear regression to generate growth models. Length-weight relationships were also analyzed with ANOVA and used as a proxy for comparing energy storage between populations.

Experiment 2

To investigate potential differences in growth rate between populations at different temperatures, spotted gars from both populations were divided into three temperature groups; 16 °C, 23 °C, and 30 °C, for a total of six groups (one peripheral group and one core group per temperature treatment). Each group was

comprised of six spotted gars for a total of 36 gars in the experiment. Fish were randomly selected from both experiment 1 as well as excess individuals, and were all reared under the same temperature (23 °C) and feeding (ad libitum) regime for at least 30 days prior to beginning the experiment.

Each group of gars was placed in a 190 L fiberglass tank containing a stand pipe connected to a large recirculating system for constant water filtration. Temperature was maintained using 75-watt heaters in the control and warm treatment group tanks, and was monitored daily. All groups were acclimated to respective temperature treatments for at least 7 days prior to beginning the experiment. Spotted gars in all tanks were given unlimited ration of fathead minnows, and photoperiod was maintained at 12 hours light/dark. Within each tank individual fish were identified by a single fin clip from the right/left pectoral fin, right/left pelvic fin, anal fin, or no fin clip. Marked fins were re-clipped as necessary (due to fin regeneration) on measurement days over the course of the experiment. Length and weight of all fish were measured at the beginning of the experiment as well as weekly for five weeks. Total duration of the experiment was 42 days.

Mean length and weight were determined for both populations in each treatment weekly, and growth rate was calculated as in experiment 1. Length-weight relationships were also calculated and analyzed for each temperature treatment and used as a proxy for energy storage similarly to experiment 1. Due to limitations in replication because of low numbers of available fish and tanks (only 1 replicate of 6 fish for each population per temperature treatment), primarily descriptive statistics were used to analyze experiment 2.

In addition to descriptive statistics, ANOVA tests were run using each fish as a replicate (N = 6 replicates per population in each treatment) to further investigate differences in growth rate and length-weight relationships between populations at each temperature. ANCOVA with temperature and population as fixed factors was performed for analysis of growth rate. All statistical analyses were carried out using JMP SAS (2001) software with significance levels set at $\alpha = 0.05$.

RESULTS

Eggs from both populations hatched 6-7 days after fertilization. Hatching success was 70-80% for both populations, and newly hatched larvae were approximately 1.0 cm in length and weighed approximately 0.5 g. Larval gars consumed their yolk sacs 6-7 DAH and began feeding on Daphnia and Artemia. Juveniles from both populations began eating small fathead minnows 35-40 DAH; 30 fish from each population were then randomly selected and moved into experimental tanks for acclimation.

Growth rates in length and weight during early life were significantly higher (ANCOVA, $p < 0.05$) for LA spotted gars than MI spotted gars held at 23 ºC (Figures 2 and 3). Length and weight regression models explained 96-99% of variation in the data. Although both groups of fish were of similar age when switching to piscivory and acclimating to experimental aquaria, 1-way ANOVA tests indicated MI fish were significantly smaller than LA fish at the beginning of experiment 1 (Table 1). One-way ANOVA tests indicated that end length and weight of MI fish, however, were significantly higher than end length and weight of LA fish. ANCOVA tests also indicated that growth rates of MI gars were significantly greater than those of LA gars. Linear regression analyses generated models of growth rates for both populations and explained 97-99% of variation in the data (Figures 4 and 5).

Length-weight relationships were compared using one-way ANOVA at the beginning and end of experiment 1; ANCOVA was used to compare rate of change in length-weight relationships during the course of experiment 1. At the beginning of experiment 1, MI fish had a significantly lower weight at a given length than LA fish. By the end of experiment 1, however, MI fish had a significantly higher weight at length than LA fish. Linear regression analysis and ANCOVA indicated that change in weight-length ratios were significantly different between MI (higher rate) and LA fish (lower rate) over the course of experiment 1 (Figure 6).

In experiment 2, both populations responded differently to temperature treatments (Table 2, Figure 7). Fish from both populations at 16 °C exhibited very low increases in length (MI fish = 0.02 cm, LA fish = 0.10 cm) and decreased in weight (MI fish = -1.18 g, LA fish = -0.38 g) during the 42-day period. Clipped fins (used to identify individual fish) did not regenerate on any individuals in either cool treatment, and consumption of fathead minnows was very low compared to other temperature treatments. Fish in the 23 °C and 30 °C treatments frequently required re-clipping of marked fins, as well as much more frequent replacement of fathead minnows. MI fish at 23 °C and 30 °C experienced larger mean increase in growth and growth rate (weight) compared to LA fish. One-way ANOVA tests comparing growth rates among all temperature treatments indicated that both populations experienced lowest growth rates at 16 °C, higher growth rates at 23 °C, and highest growth rates at 30 °C (Figure 8). Comparing growth rates within populations at different temperatures, MI fish experienced significantly higher growth in length from 16 °C to 23 °C, but not from 23 °C to 30 °C. LA fish experienced significantly higher growth in length among all three temperature treatments. MI fish experienced significantly higher growth in weight across all temperature treatments, while LA fish experienced significantly higher growth in weight from 16 °C to 23 °C, but not from 23 °C to 30 °C. The 16 °C treatment may have been near the point at which growth ceases in both populations of spotted gars.

DISCUSSION

We hypothesized that spotted gars from two disjunct population segments would exhibit latitudinal compensation in growth similar to several other fish species (Conover et al. 2009), and that under common environment conditions, fish from higher latitude would grow faster than those from lower latitude. Our experiments showed that in a common environment simulating periods within the first growing season (experiment 1: T = 23 ºC, duration approximately 60 days, 95-155 DAH; experiment 2: T = 16, 23, or 30 ºC, duration = 42 days), peripheral population spotted gars had a significantly higher growth rate than core population spotted gars, suggesting that important genetic and physiological differences exist between the two major population segments. Although lack of replication limited the extent of our statistical analyses in experiment 2, results clearly suggest that MI spotted gars maintained a higher growth rate than core population spotted gars even at warmer temperatures, and that both populations had similar thermal minima for growth. These results strongly support evidence for CnGV in growth rate in spotted gars.

As in Atlantic silversides, the model species used to investigate CnGV in growth by Conover and Present (1990) (see also Conover 1992, Present and Conover 1992, Munch and Conover 2002), spotted gars begin spawning at approximately the same temperature (23 ºC) but later in the year with increasing latitude (Redmond 1964, Holt 1973, Trautman 1981, Becker 1983, Snedden 1999). Conover et al. (1990) also noted that later initiation of spawning and earlier onset of winter resulted in a much shorter growing season at higher latitudes. Although the length of growing season decreases as latitude increases, mean size at the end of first growing season does not decrease for several populations of fish species with increasing latitude (Conover et al. 2009). Therefore populations of these species at higher latitudes are able to compensate for shorter growing seasons by evolving faster growth rates than lower-latitude populations (Conover 1992).

These differences in growth rate may be indicative of other potentially interesting eco-evolutionary dynamics between core and peripheral populations of spotted gars (explored in chapter 2) such as differences in life history patterns, as well as morphological and genetic variation. From an evolutionary ecology perspective, my results suggest that a rapid adaptation in growth rate has occurred even in relatively slowly-evolving fishes such as gars (Wiley 1976, Conover et al. 2009, Grande 2010, Carlson et al. 2011). The spotted gar, a warmwater species, entered the Great Lakes region via connections to the Mississippi River drainage (southern refugium) following the last glaciation no more than 8,000 years ago (Bailey and Smith 1981, Hocutt and Wiley 1986). Therefore adaptation of growth rate to length of growing season was relatively recent. Similarly, Mach et al. (2011) showed that in Atlantic silversides, another species expanding northward from a single southern refugium post-glaciation, regional adaptation

(e.g. CnGV) and phenotypic patterns developed relatively recently. Using Pacific salmonids, Carlson et al. (2011) showed that shifts in body size due to selection over even a single generation can have large and lasting evolutionary impacts on both species and ecosystems.

The scope of our study was limited to two major populations (core and peripheral) of spotted gars; including more populations in future experiments may provide a better picture of gradient in growth rate with increasing latitude. Despite this limitation, our study populations did represent a natural break in the distribution of spotted gars, in that the species is completely disjunct between the Great Lakes and Mississippi River basins (Page and Burr 1991), therefore our population comparisons are realistic if not comprehensive. The core population does span a greater latitudinal range than the peripheral population (approximately 1550 km compared to 220 km), therefore growth rate comparisons among fish from multiple core populations are recommended.

Although CnGV has been observed in a diversity of ectotherms, most frequently in fishes, it has not been previously observed in gars. Furthermore, our study is the first to use common garden experiments to test for latitudinal variation in a non-teleost fish; an under-studied group in such investigations, because of their typically late maturation and long generation time (Ferrara 2001), as well as high energy requirements (Alfaro et al. 2008) compared to teleosts in similar studies (Conover and Present 1990, Schultz et al. 1996, Arendt and Wilson 1997, Power and McKinley 1997, Conover et al. 2009, Baumann and Conover 2010). Our results suggest that CnGV may exist in other evolutionarily and economically significant non-teleost species (i.e. lungfishes, sturgeons, alligator gar).

Countergradient variation in growth of spotted gars may also have implications in the context of climate change and range expansion. Using the weak latitudinal temperature gradient of the Pacific silversides *Atherinops affinis* as a proxy for the gradual effects of climate change, Baumann and Conover (2010) showed that two species, Atlantic and Pacific silversides, each experiencing very different latitudinal temperature gradients, still exhibited CnGV in growth. Their study indicated that ectotherms have evolved growth adaptations to even weak climate gradients, and that a pole-ward migration of genotypes will be a likely result of an increasingly warmer climate. As a warmwater species exhibiting CnGV, spotted gars would likely successfully increase their range northward even with gradual increases in temperature.

Previous studies have shown that in aquatic systems, species at higher trophic levels are at higher risk and are more frequently lost than those at lower trophic levels, in part because of their relatively small population sizes (Lande 1993, Petchey et al. 2004). Piscivorous fishes, therefore, may be particularly vulnerable amidst the ongoing biodiversity crisis. Furthermore, non-game piscivorous species (e.g. gars, Lepisosteidae; bowfin, Amia calva) may be even more at risk due to their poorly-studied ecology, perceived low economic value, and the higher priority given to propagation and management of game species (centrarchids, percids, esocids); the latter often leading to the destruction of both non-game individuals and habitat (Scarnecchia 1992). Our study provides evidence of unique characteristics of the peripheral population of spotted gars, and provides more evidence for the general argument that understanding and protecting peripheral populations should be a key component of our programs to conserve natural biodiversity.

LITERATURE CITED

Alfaro, R.M., C.A. Gonzales, and A.M. Ferrara. 2008. Gar biology and culture: status and prospects. Aquaculture Research 39: 748-763.

Allendorf, F.W. 1988. Conservation biology of fishes. Conservation Biology 2: 145-148.

Baumann, H. and D.O. Conover. 2011. Adaptation to climate change: contrasting patterns of thermal-reaction-norm evolution in Pacific versus Atlantic silversides. Proceedings of the Royal Society B 278: 2265-2273.

Bailey, R.M. and G.R. Smith. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 38: 1539-1561.

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.

Berrigan, D. and J.C. Koella. 1994. The evolution of reaction norms: simple models for age and size at maturity. Journal of Evolutionary Biology 7: 549-566.

Billerbeck, J.M., T.E. Lankford, and D.O. Conover. 2001. Evolution of intrinsic growth and energy acquisition rates: I. tradeoffs with swimming performance in *Menidia menidia*. Evolution 55: 1863-1872.

Breck, J.E., C.P. Simon, E.S. Rutherford, P.J. Lamberson, B.S. Low, and D.R. Swank. 2008. When to mature? New Answers from life history theory, with applications to Great Lakes salmonids. *In review*.

Brooks, T.M., R.A. Mittermeier, G.A.B. da Fonesca, J. Gerlach, M. Hoffmann, J.F. Lamoreux, C.G. Mittermeier, J.D. Pilgrim, A.S.L. Rodrigues. 2006. Global biodiversity conservation priorities. Science 313: 58-61.

Carlson, S.M., T.P. Quinn, and A.P. Hendry. 2011. Eco-evolutionary dynamics in Pacific salmon. Heredity 1-10.

Carman, S.M. 2002. Special animal abstract for *Lepisosteus oculatus* (spotted gar). Michigan Natural Features Inventory. Lansing, MI. 1-3.

Channell, R. and M.V. Lomolino. 2000. Trajectories to extinction: spatial dynamics of the contraction of geographical ranges. Journal of Biogeography 27: 169-179.

Conover, D.O. 1990. The relation between capacity for growth and length of growing season: evidence for and implications of countergradient variation. Transactions of the American Fisheries Society 119: 416-430.

Conover, D.O., and T.M.C. Present. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia 83: 316-324.

Conover, D.O. and E.T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecology and Evolution 10: 248-252.

Conover, D.O., S.A. Arnott, M.R. Walsh, and S.B. Munch. 2005. Darwinian fishery science: lessons from the Atlantic silverside (*Menidia menidia*). Canadian Journal of Fisheries and Aquatic Sciences 62: 730-737.

Conover, D.O., T.A. Duffy, and L.A. Hice. 2009. The covariance between genetic and environmental influences across ecological gradients. Annals of the New York Academy of Sciences 1168: 100-129.

Convention on Biological Diversity. 2008. Available: http://www.cbd.int/. (November 2008)

Dudgeon, D., A.H. Arthington, M.O. Gessner, Z. Kawabata, D.J. Knowler, C.Leveque, R.J. Naiman, A. Prieur-Richard, D. Soto, M.L.J. Stiassny, and C.A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163-182.

Echelle, A.A., and C.D. Riggs. 1972. Aspects of the early life history of gars (*Lepisosteus*) in Lake Texoma. Transactions of the American Fisheries Society 101: 106-112.

Ferrara, A.M. 2001. Life-history strategy of Lepisosteidae: implications for the conservation and management of alligator gar. Doctoral dissertation, Auburn University, Auburn, Alabama.

Ferrara, A. M. and E. R. Irwin. 2001. Standardized procedure for internal sex identification in Lepisosteidae. North American Journal of Fisheries Management 21: 956-961.

Foster, S.J. and A.C.J. Vincent. 2004. Life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology 65: 1-61.

Garner, A., J.L. Rachlow, and J.F. Hicks. 2005. Patterns of genetic diversity and its loss in mammalian populations. Conservation Biology 19: 1215-1221.

Goodyear, C. P. 1967. Feeding habits of three species of gars, Lepisosteus, along the Mississippi gulf coast. Transactions of the American Fisheries Society 96:438- 449.

Grande, L. 2010. An empirical synthetic pattern study of gars (Lepisosteiformes) and closely related species, based mostly on skeletal anatomy. The resurrection of holostei. American Society of Ichthyologists and Herpetologists Special Publication 6: i-x, 1-871; supplementary issue of Copeia 10: 2a.

Heibo, E., C. Magnhagen, and L.A. Vollestad. 2005. Latitudinal variation in life-history traits in Eurasian perch. Ecology 86: 3377-3386.

Helfman, G.S. 2007. Fish Conservation. Island Press, Washington.

Herrington, S.J., K.N. Hettiger, E.J. Heist, and D.B. Keeney. 2008. Hybridization between longnose and alligator gars in captivity, with comments on possibly gar hybridization in nature. Transactions of the American Fisheries Society 137: 158-164.

Hocutt, C.H. and E.O. Wiley. 1986. The Zoogeography of North American Freshwater Fishes. John Wiley and Sons, New York.

Holt, R. 1973. Age and growth, length-weight relationship, condition coefficient, and feeding habits of spotted gar, *Lepisosteus oculatus* (Winchell), within the Kentucky waters of Kentucky Reservoir. Masters thesis, Murray State University, Murray, Kentucky.

Hooper, D.U., F.S. Chapin III, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H. Lawton, D.M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A.J. Symstad, J. Vandermeer, and D.A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75: 3-35.

Hubbs, C.L., K.F. Lagler, and G.R. Smith. 2004. Fishes of the Great Lakes Region, revised edition. The University of Michigan Press, Ann Arbor, Michigan.

Hurst, T.P. 2007. Causes and consequences of winter mortality in fishes. Journal of Fish Biology 71: 315-345.

Johannesson, K. and C. Andre. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Molecular Ecology 15: 2013-2029.

Johnson, B.L., and D.B. Noltie. 1996. Migratory dynamics of stream-spawning longnose gar (*Lepisosteus osseus*). Ecology of Freshwater Fish 5: 97-107.

Johnson, B.L., and D.B. Noltie. 1997. Demography, growth, and reproductive allocation in stream-spawning longnose gar. Transactions of the American Fisheries Society 126: 438-466.

Jones, B., C.Gliddon, and J.E.G. Good. 2001. The conservation of variation in geographically peripheral populations: *Lloydia serotina* (Liliaceae) in Britain. Biological Conservation 101: 147-156.

Kyle, C.J. and C. Strobeck. 2002. Connectivity of peripheral and core populations of North American wolverines. Journal of Mammalogy 83: 1141-1150.

Kynard, B. 1997. Life history, latitudinal patterns, and status of the shortnose sturgeon, *Acipenser brevirostrum*. Environmental Biology of Fishes 48: 319-334.

Lammi, A., P. Siikamaki, and K. Mustajarvi. 2001. Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. Conservation Biology 13: 1069-1078.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist 142: 911-927.

Lardies, M.A., L.D. Bacigalupe, and F. Bozinovic. 2004. Testing the metabolic cold adaptation hypothesis: an intraspecific latitudinal comparison in the common wood louse. Evolutionary Ecology Research 6: 567-578.

Latta, W.C. 2003. Distribution and abundance of Michigan fishes collected 1993-2001. Michigan Department of Natural Resources, Fisheries Research Report, Ann Arbor.

Lee, H., D. DeAngelis, and H. Koh. 1998. Modeling spatial distribution of the unionid mussels and the core-satellite hypothesis. Water, Science, and Technology 38: 73-79.

Lesica, P. and F.W. Allendorf. 1995. When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Love, J.W. 2002. Sexual dimorphism in spotted gar *Lepisosteus oculatus* from southwestern Louisiana. American Midland Naturalist 147: 393-399.

Love, J.W. 2004. Age, growth, and reproduction of spotted gar, *Lepisosteus oculatus* (Lepisosteidae), from the Lake Pontchartrain estuary, Louisiana. The Southwestern Naturalist 49: 18-23.
Luck, G.W., G.C. Daily, and P.R. Ehrlich. 2003. Population diversity and ecosystem services. Trends in Ecology and Evolution 18: 331-336.
Mach, M.E., E.J. Sbrocco, L.A. Hice, T.A. Duffy, D.O. Conover, and P.H. Barber. 2011. Regional differentiation and post-glacial expansion of the Atlantic silverside, *Menidia menidia*, an annual fish with high dispersal potential. Marine Biology 158: 515-530.
Mandrak, N.E. and E.J. Crossman. 1992. Postglacial dispersal of freshwater fishes into Ontario. Canadian Journal of Zoology 70: 2247-2259.
Marcil, J., D.P. Swain, and J.A. Hutchings. 2006. Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.). Biological Journal of the Linnean Society 88: 351-365.
Michigan Department of Natural Resources. 2005. Species of Greatest Conservation Need. Michigan's Wildlife Action Plan. Available: http://www.michigandnr.com/publications/pdfs/huntingwildlifehabitat/wcs/sgcn. (March 2008)
Munch, S.B., M. Mangel, and D.O. Conover. 2003. Quantifying natural selection on body size from field data: winter mortality in *Menidia menidia*. Ecology 84: 2168-2177.
National Oceanic and Atmospheric Administration National Climatic Data Center. 2011. U.S. Climate Normals 1971-2000. Available: http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl. (February 2011)
Nelson, J. S. 1994. Fishes of the world, 3rd edition. Wiley, New York.
Netsch, N.F., and A. Witt, Jr. 1962. Contributions to the life history of the longnose gar, (*Lepisosteus osseus*) in Missouri. Transactions of the American Fisheries Society 91: 251-262.
Page, L.M., and B.M. Burr. 1991. A Field Guide to Freshwater Fishes. Houghton Mifflin Company, Boston.
Petchey, O.L., A.L. Downing, G.G. Mittelbach, L. Persson, C.F. Steiner, P.H. Warren, and G. Woodward. 2004. Species loss and structure and functioning of multitrophic aquatic systems. Oikos 104: 467-478.
Pope, K.L. and G.R. Wilde. 2003. Variation in spotted gar (*Lepisosteus oculatus*) mass-length relationships in Texas reservoirs. Texas Journal of Science 55: 43-48.
Power, M. and R.S. McKinley. 1997. Latitudinal variation in lake sturgeon size as related to the thermal opportunity for growth. Transactions of the American Fisheries Society 126: 549-558.
Redmond, L. C. 1964. Ecology of the spotted gar (*Lepisosteus oculatus* Winchell) in Southeastern Missouri. Master's thesis, University of Missouri, Columbia, Missouri.

Robertson, C.R., S.C. Zeug, and K.O. Winemiller. 2007. Associations between hydrological connectivity and resource partitioning among sympatric gar species (Lepisosteidae) in a Texas river and associated oxbows. Ecology of Freshwater Fish 17: 119-129.
Scarnecchia, D. L. 1992. A reappraisal of gars and bowfins in fishery management. Fisheries 17: 6-12.
Scudder, G.G.E. 1989. The adaptive significance of marginal populations: a general perspective. *in*: Proceedings of the national workshop on effects of habitat alteration on salmonid stocks. C.D. Levings, L.G.Holtby, and M.A. Henderson, editors. Canadian Special Publication of Fisheries and Aquatic Science 105: 180-185.
Simon, T. P. and R. Wallus.1989. Contributions to the early life histories of gar (Actinopterygii: Lepisosteidae) in the Ohio and Tennessee River basins with emphasis on larval development. Transactions of the Kentucky Academy of Science 50:59-71.
Simon, T. P. and E. J. Tyberghein. 1991. Contributions to the early life history of the spotted gar, *Lepisosteus oculatus* Winchell, from Hatchet Creek, Alabama. Transactions of the Kentucky Academy of Science 52:124-131.

Slaughter IV, J.E., R.A. Wright, and D.R. DeVries. 2004. The effects of age-0 body size and the predictive ability of a largemouth bass bioenergetics model. Transactions of the American Fisheries Society 133: 279-291.

Slaughter IV, J.E., R.A. Wright, and D.R. DeVries. 2008. Latitudinal influence on first-year growth and survival of largemouth bass. North American Journal of Fisheries Management 28: 993-1000.

Smith, G.R. 1981. Late Cenozoic freshwater fishes of North America. Annual Reviews of Ecology and Systematics 12: 163-193.

Snedden, G.A., W.E. Kelso, and D.A. Rutherford. 1999. Diel and seasonal patterns of spotted gar movement and habitat use in the lower Atchafalaya River Basin, Louisiana. Transactions of the American Fisheries Society 128: 144-154.

Stearns, S.C. and J.C. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40: 893-913.

Suttkus, R. D. 1963. Order Lepisostei. *In* Fishes of the Western Atlantic; Part Three, Soft-rayed fishes. Yale University Memoir Sears Foundation for Marine Research 1, eds. H.B. Bigelow et al., 61-88. New Haven, Connecticut.

Trautman, M.B. 1981. The Fishes of Ohio, revised edition. Ohio State University Press, Columbus.

Tyler, J. D. and M. N. Granger. 1984. Notes on the food habits, size, and spawning behavior of spotted gar in Lake Lawtonka, Oklahoma. Proceedings of the Oklahoma Academy of Sciences 64:8-10.

U.S. Environmental Protection Agency. 2007. Biological Indicators of Watershed Health. Available: www.epa.gov/bioiweb1/html/fish_indicators.html. (August 2008).

Wiley, E. O. 1976. The phylogeny and biogeography of fossil and recent gars (Actinopterygii: Lepisosteidae). University of Kansas Museum of Natural History Miscellaneous Publication 64:1-111.

Wisely, S.M., S.W. Buskirk, G.A. Russell, K.B. Aubry, and W.J. Zielinski. 2004. Genetic diversity and structure of the fisher (*Martes pennanti)* in a peninsular and peripheral metapopulation. Journal of Mammalogy 85: 640-648.

Wilson, B.S. and D.E. Cooke. 2004. Latitudinal variation in rates of overwinter mortality in the lizard *Uta stansburiana*. Ecology 85: 3406-3417.

Yamahira, K. and D.O. Conover. 2002. Intra- vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology 83: 1252-1262.

Table 1. Mean length (cm) and weight (g) at initiation and completion of experiment 1, along with total growth (Final-Initial), growth rate ($cm \cdot day^{-1}$, $g \cdot day^{-1}$), and descriptive statistics for LA and MI populations of spotted gars (N=30 fish per population). Experimental durations were 62 (LA) and 63 (MI) days.

Population	**Michigan**			**Louisiana**		
	Mean	Variance	St Dev	Mean	Variance	St Dev
Initial Length	14.29	3.41	1.85	15.56	2.40	1.55
Final Length	20.06	2.70	1.64	18.24	2.11	1.45
Total Growth	5.77			2.68		
Growth Rate	0.09			0.04		
Initial Weight	7.50	9.07	3.01	10.74	10.33	3.21
Final Weight	24.09	46.80	6.84	17.53	16.93	4.12
Total Growth	16.59			6.79		
Growth Rate	0.26			0.11		

Table 2. Mean length (cm) and weight (g) at initiation and completion of experiment 2, along with total growth (Final-Initial), growth rate ($cm \cdot day^{-1}$, $g \cdot day^{-1}$), and descriptive statistics for LA and MI populations of spotted gars at 3 different temperature treatments (N = 6 fish per population in each treatment). Experimental duration was 42 days.

Experimental Temperature (°C)		Michigan			Louisiana		
			Length				
		Mean	Variance	St Dev	Mean	Variance	St Dev
16	Initial Length	21.07	1.05	1.03	19.17	1.59	1.26
	Final Length	21.08	0.95	0.97	19.27	1.87	1.37
	Total Growth	0.02			0.10		
	Growth Rate	< 0.01			< 0.01		
23	Initial Length	20.75	0.67	0.82	19.85	7.30	2.70
	Final Length	23.52	1.26	1.12	21.33	7.37	2.72
	Total Growth	2.77			1.48		
	Growth Rate	0.07			0.04		
30	Initial Length	22.60	6.86	2.62	20.72	0.90	0.95
	Final Length	25.50	2.96	1.72	23.15	1.24	1.11
	Total Growth	2.90			2.43		
	Growth Rate	0.07			0.06		
			Weight				
		Mean	Variance	St Dev	Mean	Variance	St Dev
16	Initial Weight	27.73	22.41	4.73	21.77	24.25	4.92
	Final Weight	26.55	18.58	4.31	21.38	25.16	5.02
	Total Growth	-1.18			-0.38		
	Growth Rate	-0.03			-0.01		
23	Initial Weight	24.63	10.61	3.26	23.73	96.48	9.82
	Final Weight	37.12	44.87	6.70	30.05	195.19	13.97
	Total Growth	12.48			6.32		
	Growth Rate	0.30			0.15		
30	Initial Weight	32.50	134.49	11.60	25.27	20.06	4.48
	Final Weight	51.97	108.97	10.44	36.32	41.93	6.48
	Total Growth	19.47			11.05		
	Growth Rate	0.46			0.26		

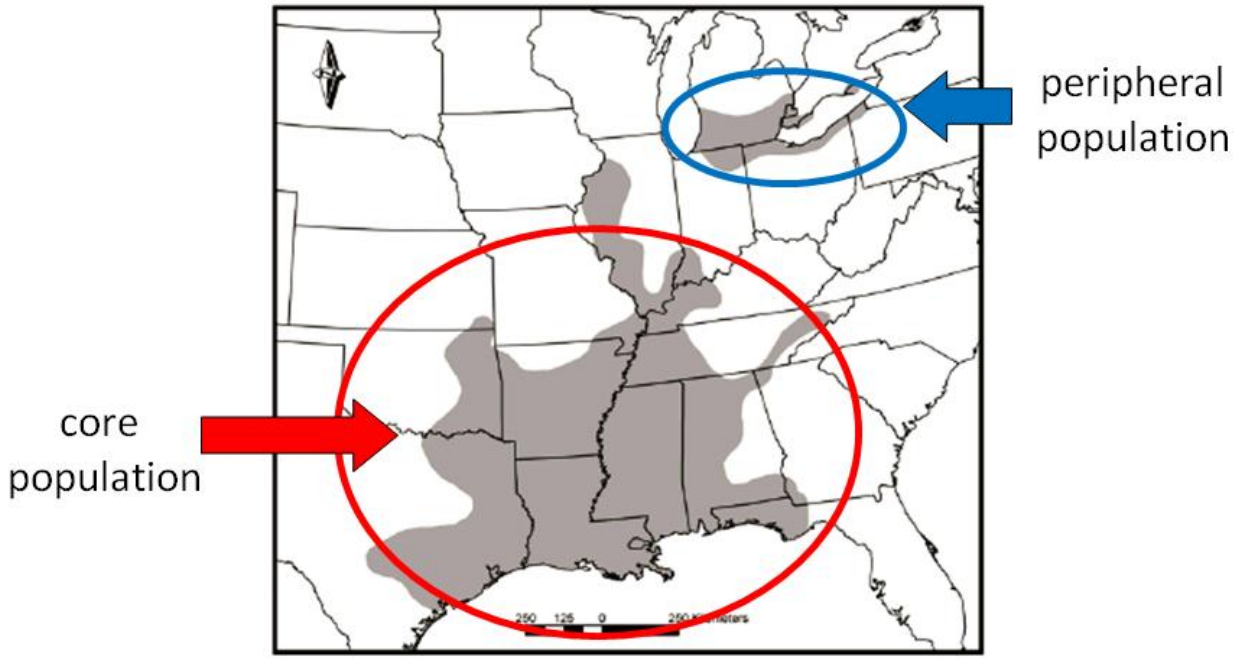


Figure 1. Distribution of core and peripheral populations of the spotted gar *Lepisosteus oculatus*. Note disjunction between populations. Modified from Page and Burr (1991).

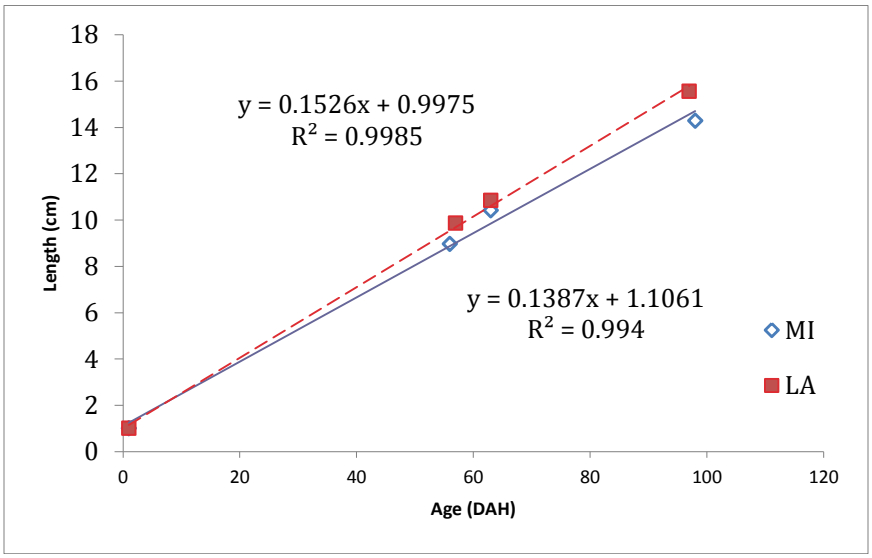


Figure 2. Comparison of early life stage length at age (period prior to start of experiment 1) of LA and MI populations of spotted gars held at 23 ºC (N = 30 fish per population). Larval fish from both populations hatched at approximately 1.0 cm. Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

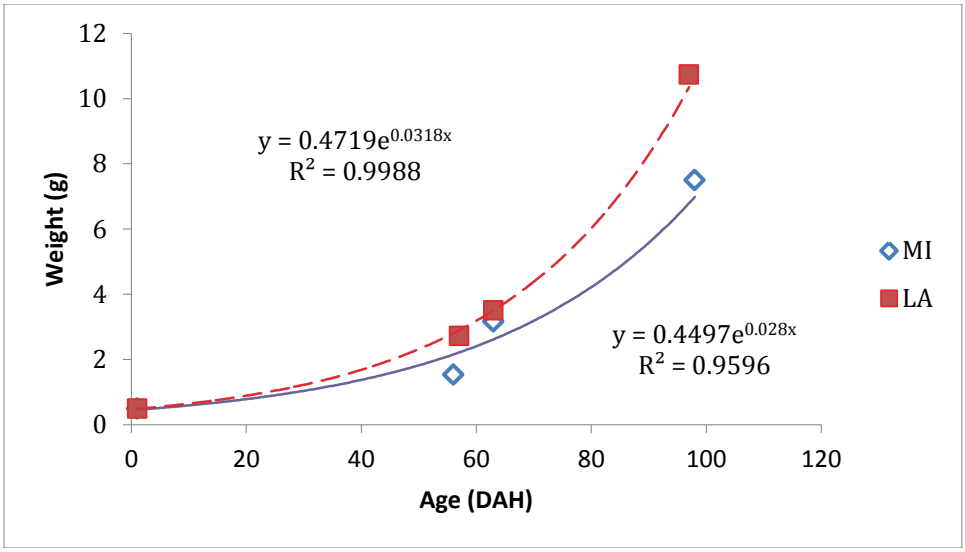


Figure 3. Comparison of early life stage weight at age (period prior to start of experiment 1) of LA and MI populations of spotted gars held at 23 ºC (N = 30 fish per population). Larval fish from both populations hatched at approximately 0.5 g. Exponential regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

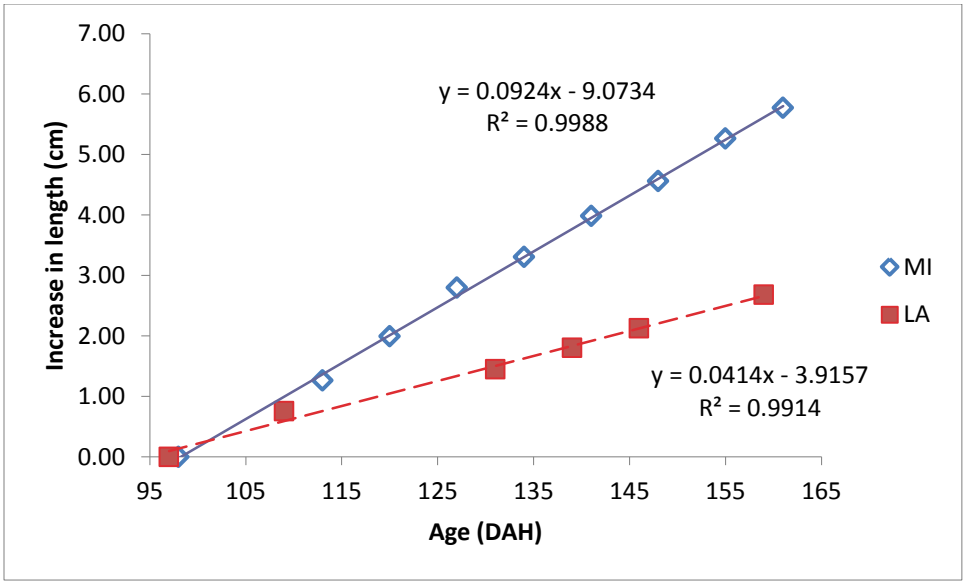


Figure 4. Increase in length over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

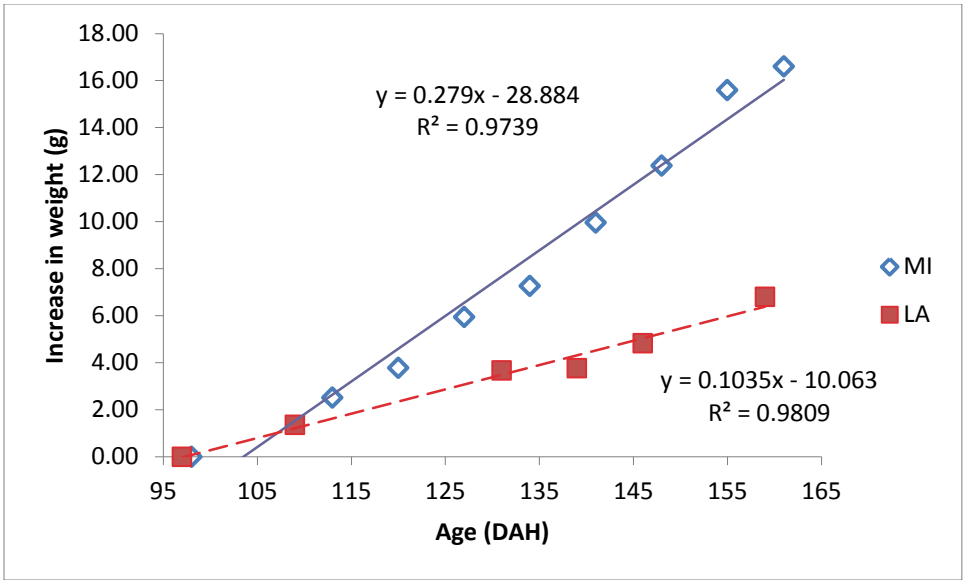


Figure 5. Increase in weight over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

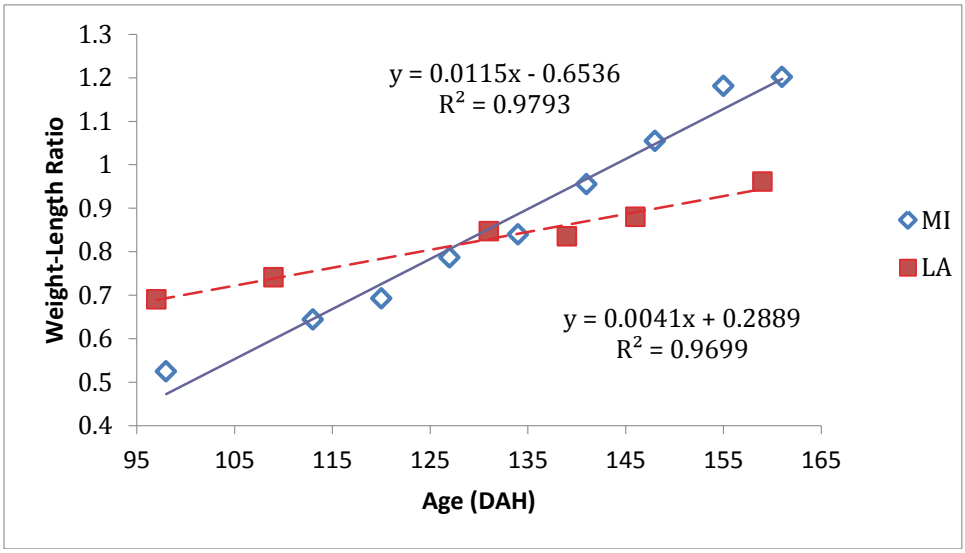


Figure 6. Mean weight-length ratios over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

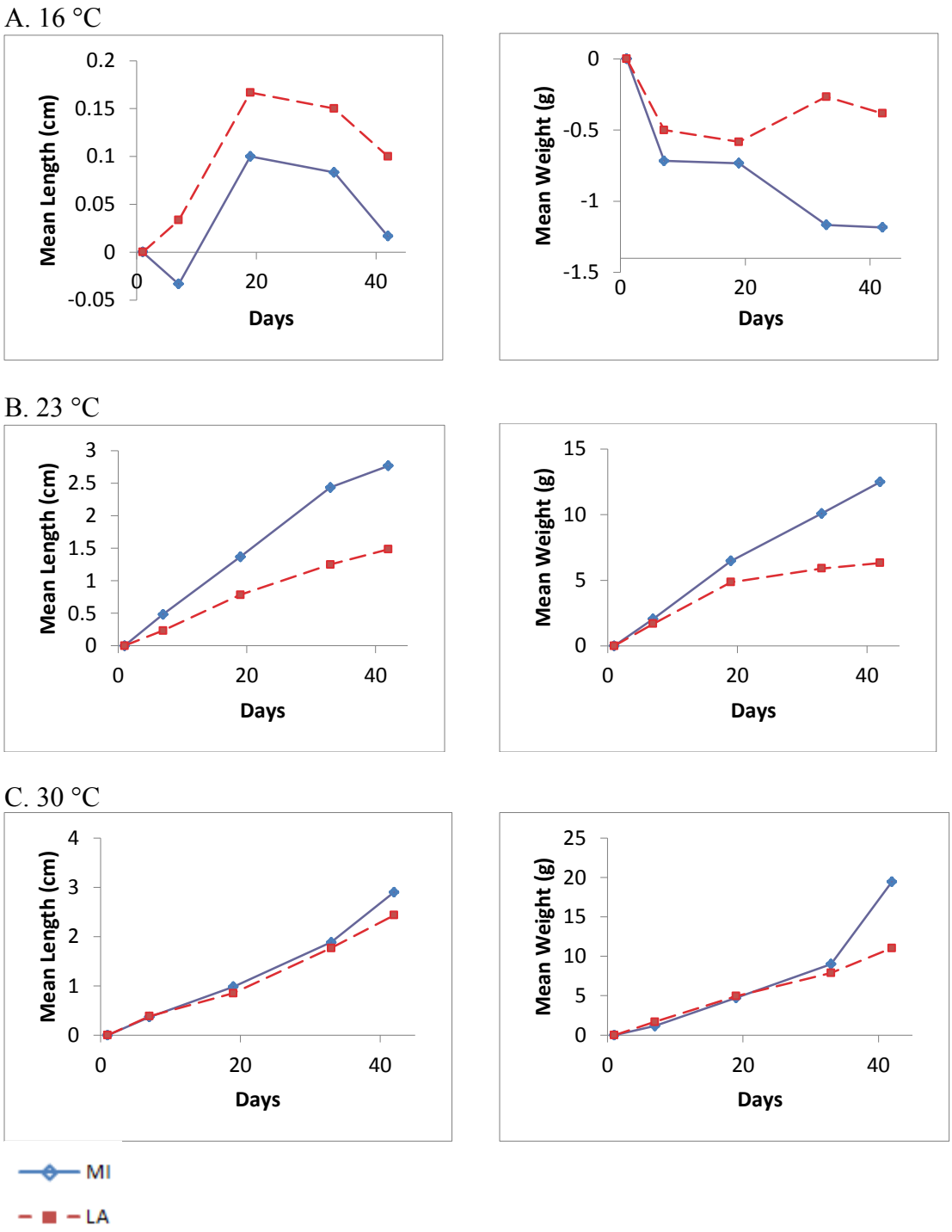
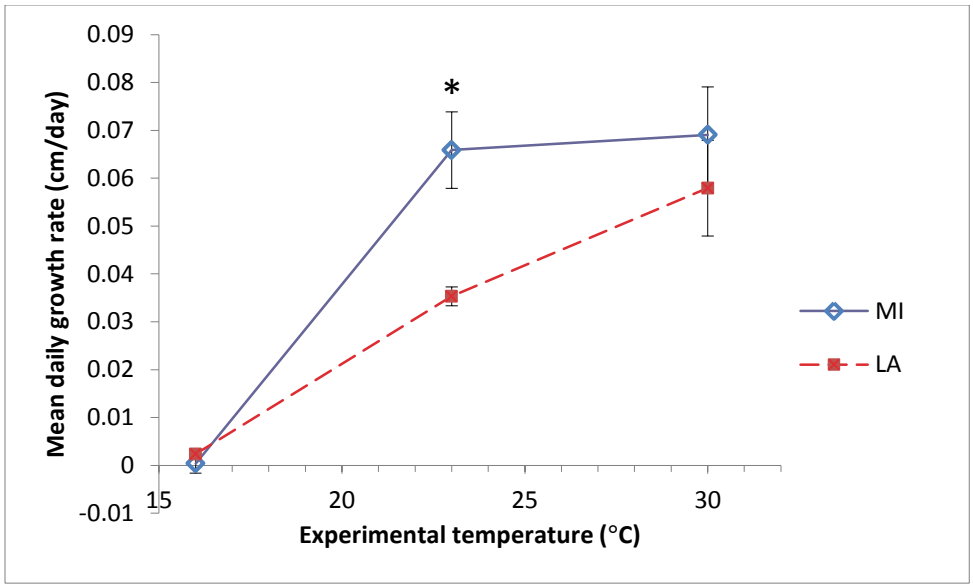


Figure 7. Changes in mean length and weight for MI (solid line) and LA (dashed line) populations of spotted gars at 3 temperature treatments (A = 16 °C, B = 23 °C, C = 30 °C; N = 6 fish per population in each treatment) in experiment 2 (experimental duration = 42 days).



B.

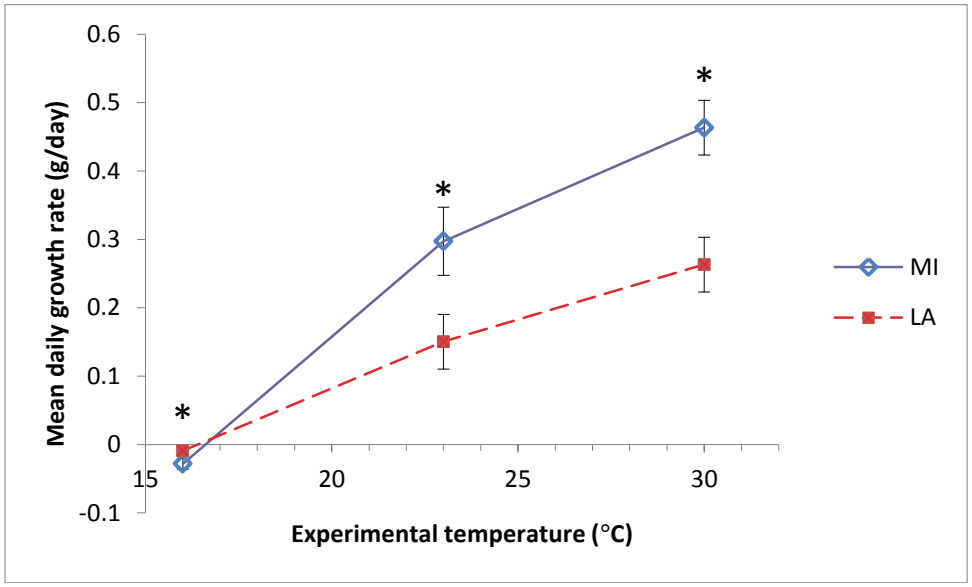


Figure 8. Mean daily growth rates for length (A) and weight (B) of LA and MI populations of spotted gars at three temperature treatments (16 °C, 23 °C, 30 °C; N = 6 fish per population in each treatment) in experiment 2 (experimental duration = 42 days). Error bars indicate ±1 standard error, * indicates significant difference between populations at temperature treatment.

Developing Hatchery Methods for the Mangrove Oyster, *Crassostrea corteziensis* for the Pacific Coast of Mexico

Indigenous Species Development/Study/09IND01UH

Eladio Gaxiola Camacho
Autonomous University of Sinaloa
Culiacán, Sinaloa, Mexico

Guillermo Rodriguez Dominguez and Olga Olivia Zamudio Armenta
Autonomous University of Sinaloa
Mazatlán, Sinaloa, Mexico

Maria Haws
University of Hawai'i at Hilo
Hilo, Hawai'i, USA

John Supan
Louisiana State University
Baton Rouge, Louisiana, USA

Graduate Students:
Luis Javier Álvarez Bajo, Gilberto Hernández Llamas, José Evaristo Varela Valenzuela and Rosalba Nayely Perea Juárez
Autonomous University of Sinaloa
Culiacán, Sinaloa, Mexico

ABSTRACT

Crassotrea corteziensis is an important oyster culture species in the Mexican State of Nayarit where it forms the basis of a thirty-year old industry. This industry is based on spat collection, which has been primarily successful only in the State of Nayarit. This limits its potential for other areas of Pacific Mexico. This investigation was designed to develop the capacity and methods to reliably produced eye-larvae in a hatchery based at the School of Marine Sciences (FACIMAR) at the Autonomous University of Sinaloa, Mazatlan Campus. This hatchery and associated training and demonstration farm would allow FACIMAR to conduct research and provide training to a wide range of stakeholders.

Most of the objectives of the investigation were achieved, including development of a successful microalgae production facility and successful broodstock conditioning system. High mortality of the larvae occurred due to bacterial and protozoan infections, which prevented production of spat for the community groups involved in this work. The bacterial infections are believed to be associated with water pollution in the waters surrounding Mazatlan. Work will continue to improve the water treatment system at FACIMAR and larviculture will be attempted again in early 2012.

INTRODUCTION

Mexican aquaculture is focused on shrimp culture, although diversification of aquaculture has been identified as a national development priority. Bivalves offer a multitude of possibilities to diversify aquaculture if specific obstacles can be addressed. Oyster production is currently stable, producing

between 40,000 to 50,000 tons annually with the Gulf of Mexico industry based on *Crassostrea virginica* representing 90% of this (CONAPESCA 2009).
Oyster production on the Pacific Coast of Mexico depends on two introduced species, the Pacific Oyster (*Crassostrea gigas*) and more recently, the Kumamoto Oyster (*C. sikamea*). Both species readily adapt to local conditions and grow rapidly. Much of the seed for these species is imported from the United States, and local seed production has been decreasing due to disease and temperature issues (CIAD 2003; Tapia et al. 2008). *C. gigas* also suffers from production issues related to its tendency to become "spawny" or "thin" during the reproductive and post-reproductive season. Mortalities also occur during the warmer months and may be related to the "summer mortality" phenomena observed for this species in the Pacific Northwest of the United States.

C. corteziensis, on the other hand, does not suffer from these issues and has a high level of local acceptance. It has been cultured primarily in Nayarit State for over 30 years but expansion of the industry locally, and to other areas in Mexico, is hampered by lack of hatchery production. This species has been produced on a limited scale in a few hatcheries previously, but techniques were considered proprietary information.

The objectives of this investigation were therefore to develop methods to condition *C. corteziensis* broodstock and for hatchery production of eye-larvae. This also included establishing a microalgae production facility at FACIMAR/UAS and training staff and students in all methods. It was also hoped that a small demonstration farm could be established in the waters adjacent to the FACIMAR facilities for research and training. If successful, this would enable FACIMAR staff to produce larvae for research purposes, and to supply small community-based farms. Training courses and student classes would utilize these facilities. Methods would also be shared with several stakeholders, including a cooperative shrimp hatchery, to encourage private sector development of oyster hatcheries.

METHODS

Development of the hatchery

The School of Marine Sciences (FACIMAR) at UAS is an education institution conducting research, extension and academic training in marine sciences and aquaculture. It is located in Mazatlan, Sinaloa, Mexico. The facility is adjacent to the main seawall of the city (Figure 1). Three small laboratory facilities were developed at FACIMAR: 1) hatchery; 2) microalgae production facility; and 3) a broodstock conditioning system.

Study 1: Evaluation of the adequacy of adjacent water areas for holding oysters.

Juvenile *C. corteziensis* were obtained from farms and distributed in mesh bags, which were hung on a long line in the area adjacent to FACIMAR to determine if these conditions were adequate for conditioning and maintaining broodstock (Figure 2). Growth was monitored for 5 months. Water quality parameters such as temperature, salinity, pH and turbidity were also monitored for the same period of time. Bacteriological analysis was also conducted for *Vibrio* and coliform bacteria in oyster tissues and water.

Collection and maintenance of oyster broodstock

Approximately 100 adult oysters were collected from different locations and kept in the oyster conditioning system at FACIMAR. This consisted of recirculation tanks with salinity at 30 ‰ and temperature at 24 ºC (same as the collection sites). Aeration was also provided. Feed consisted of three species of microalgae, *Isochrysis sp., Tetraselmis* sp. and *Chaetoceros sp*. These were supplied in three daily rations of 200,000 cells per ml, supplemented with a 0.5 g daily ration of mixed corn starch and rice flour. The tanks were cleaned before the first feeding of the day and the entire system was cleaned weekly. Figure 3 shows aspects of the broodstock and microalgae systems.

Figure 1. Location of the School of Marine Sciences (FACIMAR) in Mazatlan, Sinaloa, Mexico. Arrows indicate the location of the hatchery and oyster holding areas.

Figure 2. Transplanting of oyster broodstock to oyster holding area near FACIMAR (a and b); students and staff measuring oysters (c and d).

Figure 3. Collecting broodstock (a), recirculation system for holding broodstock (b), microalgae culture in 20 liter containers (c) and larger batch culture (d).

Microalgae culture

Microalgae cultures were started in test tubes and the volume was scaled up to 20 liters, using standard algae culture methods. The 20 liter cultures were used to inoculate 900 liter semi-batch culture vessels (Hoff and Snell 1987) (Figure 3).

Spawning

Two methods were used for spawning: thermostimulation and strip spawning. The first utilized rapid temperature changes varying between 18 and 20 ºC, with changes every 30 minutes. When oysters began to spawn, they were separated into separate containers with clean, filtered seawater. When spawning was completed, sperm was added to the eggs for fertilization. When broodstock failed to respond to thermostimulation, these were sacrificed, opened and the gonadal tissue excised using gentle scraping with a scalpel.

Larviculture

D-stage larvae were transferred into 200 liter tanks filled with filtered seawater at an initial density of 8 larvae per ml. Feed consisted of *Chaetoceras* sp. (2 million cells/ml/day). Light aeration was also supplied. Larvae were sieved out every forty-eight hours, the tanks cleaned and the water was exchanged (Figure 4).

Figure 4. Induction of spawning (e and f); sieving larvae (g and h) and microscopic vie of D-stage larvae (i).

RESULTS

Evaluation of oyster holding area in adjacent waters

The oyster kept in waters adjacent to FACIMAR showed no significant growth either in length, width or weight (Figures 5, 6 and 7). Weight was maintained at 12.5 g and length at 4.4 cm over the five month period. Survival was low at 82% of the initial count after 5 months of holding.

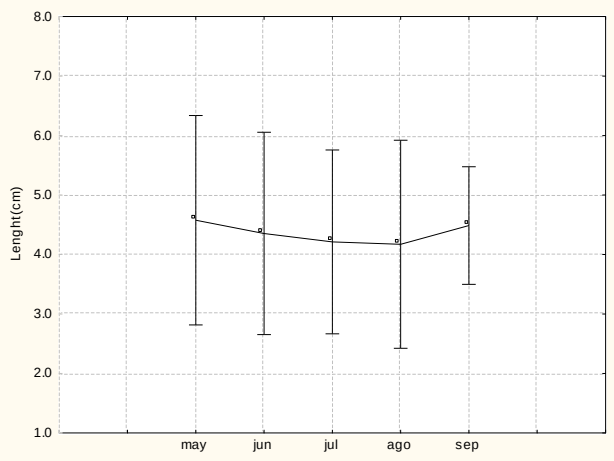


Figure 5. Monthly mean lengths (DVM) for oysters held near FACIMAR.

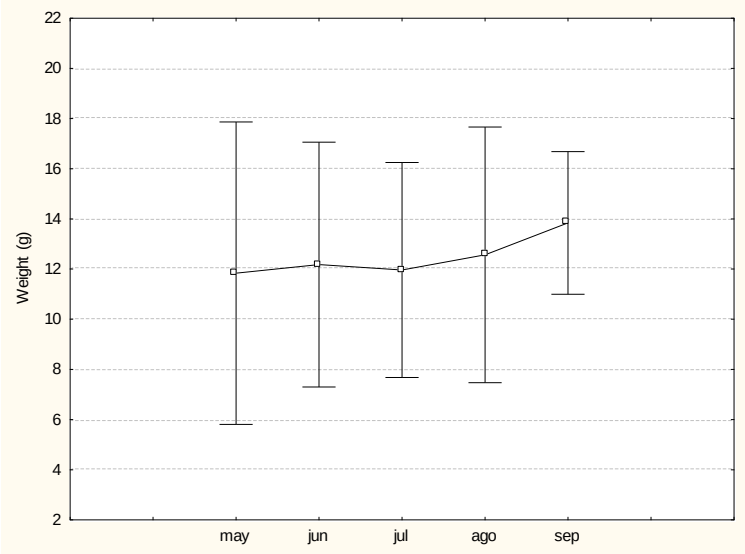


Figure 6. Monthly mean weights for oysters held near FACIMAR.

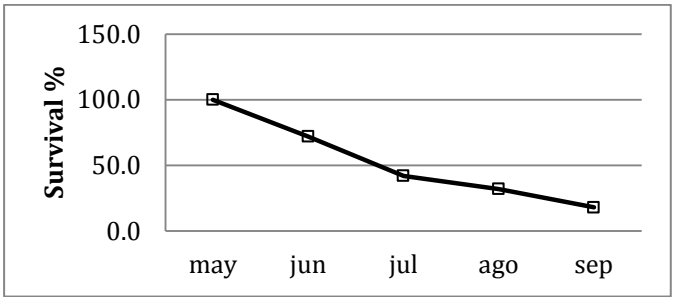


Figure 7. Monthly survival of oysters held near FACIMAR.

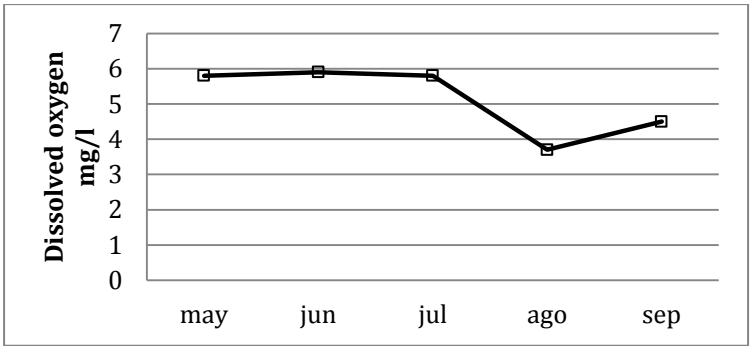


Figure 8. Dissolved oxygen levels at FACIMAR oyster holding area.

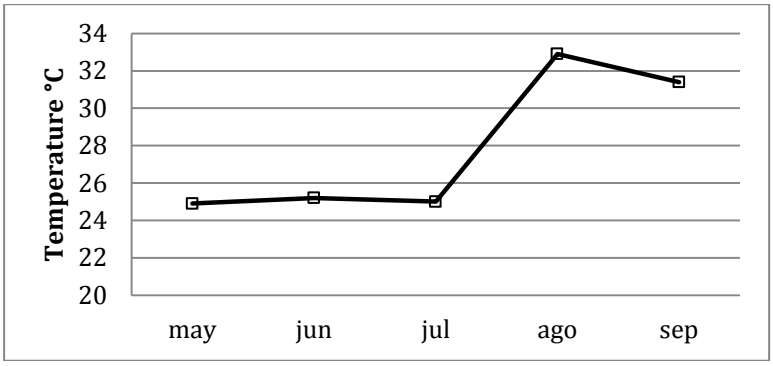


Figure 9. Mean monthly sea surface temperature at FACIMAR oyster holding area.

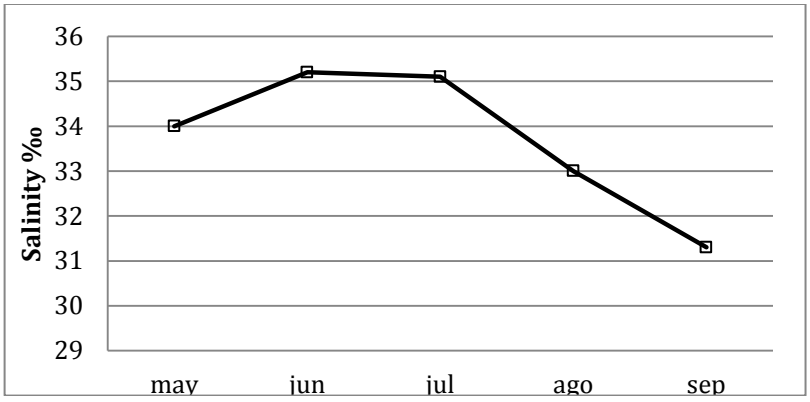


Figure 10. Mean monthly salinities for FACIMAR oyster holding area.

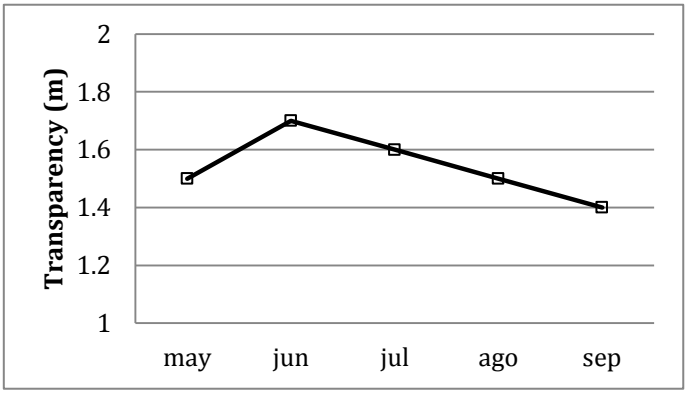


Figure 11. Mean monthly turbidity at FACIMAR oyster holding area.

Dissolved oxygen levels were lowest in August (3.7 mg/l) and September (4.5 mg/l) (Figure 8). From May to July, water temperatures were stable at 25 °C, and then rose in August and September. The maximum temperature occurred in August (32.9 °C) (Figure 9). Salinity rose from May to June (35.3‰) then declined to 31.3 ‰ in September (Figure 10). Turbidity had a mean value of 1.5 m during the five month period (Figure 11).

Vibrios and coliform bacteria in oyster tissues and seawater

Table 1 shows Vibrio values for oyster tissues and the seawater at the FACIMAR holding area. Yellow colonies were particularly prevalent.

Table 1. Results of Vibrio analysis

		UFC/ g in oyster tissues		UFC/ml in seawater	
Month	**Sample no.**	**Yellow**	**Green**	**Yellow**	**Green**
MAY	1	60333	265833	167	0
	2	333	0	0	0
JUN	1	0	333	0	0
	2	5000	2333	0	0
	3	0	0	0	0
JUL	1	333	167	25000	0
	2	2167	0	0	0
	3	0	0	0	0
AGU	1	167	0	0	0
	2	0	0	167	0
	3	0	0	1167	0
SEP	1	0	0	0	0
	2	52500	0	X	X

Table 2 shows results from testing for coliform bacteria (total and fecal) in oyster tissues and seawater. Most of the higher counts exceed the regulatory standards for acceptable limits (NOM031-SSA1-199) in shellfish growing areas.

Broodstock conditioning and maintenance

Broodstock conditioning in the recirculation system was successful before and after spawning. The mixed microalgae diet of 200,000 cells/ml along with a 0.5 g supplement of corn starch and rice flour was adequate to achieve maturation of gametes. The temperature of 23 °C and 25 ‰ salinity also appeared to be adequate.

Larviculture

Although larvae were consistently produced using thermostimulation to induce spawning, several attempts to raise larvae to the setting stage all failed with mass mortalities of larvae observed concurrently with the appearance of protozoans and other ciliated organisms. Larvae also showed signs of bacterial infections.

Table 2. Total and fecal coliform bacteria in seawater and oyster tissues.

		Seawater		Oyster tissues	
Month	Sample no.	NMP/100ml Total	NMP/100 ml Fecal	NMP/100 g Total	NMP/100 g Fecal
MAY	1	>2400	15	>2400	0
	2	215	20	507	98
JUN	1	>2400	2050	>2400	>2400
	2	>2400	45	>2400	0
	3	850	>2400	>2400	>2400
JUL	1	70	70	721	298
	2	1750	1750	426	315
	3	>2400	70	>2400	375
AGU	1	1100	>2400	482	129
	2	1750	35	>2400	>2400
	3	110	25	>2400	192
SEP	1	>2400	>2400	1345	350
	2	X	X	>2400	>2400

DISCUSSION

The high mortality rate and lack of growth exhibited by the oysters kept at the FACIMAR oyster holding area demonstrate that this site has inadequate conditions to maintain oysters. Hence, establishment of a training and research farm at this site is not possible. Given that the temperatures were within the range considered conducive to the species (21 to 31 °C) and that salinities (31 to 39‰) were also within the documented tolerances (Gongora Gomez et al. 2006), lack of adequate food may be to blame. *C. corteziensis* is an estuarine species and needs a high level of phytoplankton to thrive (Chavez Villalba et al. 2008). The mean secchi disk reading of 1.5 m suggests that this water has low levels of primary productivity (Fench et al. 1982; Shapiro et al. 1975).

An additional indication of the poor water quality of the holding area is the high levels of *Vibrios* and coliform bacteria. While species level identification was not possible, certain colony colors often indicate well known groups. In particular, the predominance of yellow colonies of *Vibrio*s are indicative of species which can harm oysters or affect humans (e.g. *V. alginolyticus*, *V. tubiashii*, *V. fluvialis*). Green colonies may include *V. parahaemolyticus, V. vulnificus, V. damsel* and *V. mimicus.* The first two can be injurious to humans. Vibriosis in general is well known to be a cause of mass mortalities in bivalve larvae hatcheries (Caceres-Martinez and Vazquez-Yeomans 2001).

The failure to produce larvae to setting stage is discouraging, but further attempts to improve water treatment to eliminate bacterial sources of contamination in the source water, as well as to eliminate contamination of the algae cultures will continue in early 2012. The basic facilities and capacity to operate a small research hatchery were successfully established during this work. Efforts will also be made to locate another location for the research and demonstration farm. Four CRSP-sponsored students were also trained as part of this research.

LITERATURE CITED

Cáceres-Martínez, J. y R. Vázquez Yeomans. 2001. Manual de enfermedades de moluscos. Boletín del Programa Nacional de Sanidad Acuícola y la Red de Diagnóstico. CONAPESCA. Año 4, Volumen 4, Número 16.

Chavez-Villalba J. A. Hernandez-Ibarra, M. R. Lopez-Tapia, and J. M. Mazon Suastegui, 2008. Prospective culture of the Cortez oyster *Crassostrea corteziensis* from northwestern Mexico: growth, gametogenic activity, and condition index. Journal of Shellfisheries Research Vol. 27, No. 4, 711–720.

CIAD. 2003. Manual de Buenas Prácticas de Producción Acuícola de Moluscos Bivalvos para la Inocuidad Alimentaria. Centro de Investigación en Alimentación y Desarrollo. Mazatlán, Sinaloa.

CONAPESCA, 2009. *Anuario estadístico de pesca 2009.* México, Secretaría de Agricultura, Ganadería, Pesca y Alimentación (www.sagarpa.gob.mx/conapesca/ index.htm).

French, R. H.; J. J. Cooper y S. Vigg. 1982. Secchi disc relationships. *Water Res. Bull.,* 18: 12 1-123.

Hoff, H. F. y T. W. Snell. 1987. Plankton Culture Manual. 5th Edition. Florida Aqua Farms, Inc. Dade City, Florida 162 p.

Tapia Vázquez, O.; H. M. González A.: L. M. Sáenz G. y R. García H. 2008. Manual de en granjas ostrícolas de San Quintín, Baja California, México. Comité Estatal de Sanidad Acuícola de Baja California A.C. 36 p.

Wyer M.D., Kay D., Jackson G.F., Dawson H.M., Yeo J. y Tanguy L. 1995. Indicator organism sources and coastal water quality: a catchment study on the island of Jersey. J. Appl. Bacteriol

Shapiro, J.; J. B. Lundquist & R. E. Carlson. 1975. Involving the public in limnology - an approach to communication. *Verh. Internat. Verein. Limnol.,* 19: 866-874.

Sustainable Snakehead Aquaculture Development in the Lower Mekong River Basin of Cambodia and Vietnam

Indigenous Species Development/Study/09IND02UC

Part 1: Breeding and Weaning of Striped Snakehead (*Channa Striata*) in Cambodia

So Nam
Inland Fisheries Research and Development Institute
Phnom Penh, Cambodia

Sam Narith
Freshwater Aquaculture Research and Development Center
Prey Veng Province, Cambodia

Bui Minh Tam and Tran Thi Thanh Hien
Can Tho University
Can Tho, Vietnam

Robert S. Pomeroy
University of Connecticut–Avery Point
Groton, Connecticut, USA

INTRODUCTION

Cambodia aquaculture represents about 10% of the total fisheries production (So Nam & Touch Bauntheng, 2011), while the Mekong delta in Vietnam nearly 60% (Le Xuan Sinh and Pomeroy, 2009). They have expanded, diversified and intensified; their contributions to aquatic food production have increased gradually and potentially. They are highly diverse and consist of a broad spectrum of systems, practices and operations, ranging from simple backyard small, household pond systems to large-scale, highly intensive, commercially oriented practices. The annual growth rate of aquaculture production in Cambodia is approximately 20% for the past decade (2001-2010) (So Nam & Touch Bunthang, 2011). According to a study conducted in FiA data 3,257 farmers own 16,547 cages representing 52% of the total production. Most of the freshwater cages are in Kandal (79%) with more than 12,000 cages. Other provinces around the Tonle Sap Lake and Mekong River have a lower number of cages, between 400 and 600 cages per province: Kampong Cham (4%), Siem Reap (4%), Pursat (3%), Battambang(3%), Kampong Chhnang (2%). *Pangasius* (in monoculture or polyculture associated with catfish, *Leptobarbus hoevenii* or carps and tilapia) and snakehead are the dominant species accounting for more than 90% of the cages. There are 40,479 earthen ponds owned by 52,284 farmers in the whole country. Most of the ponds are in southern part of Cambodia: Takeo province (19,046 ponds), Svay Rieng (9,315 ponds), Prey Veng (5,398 ponds), and Kampong Speu (3,986 ponds). Other provinces around Tonle Sap Lake have a lower number of ponds, between 250 and 1,100 ponds per province (So Nam & Srun Lim Song, 2011). *Pangasius*, hybrid catfish, snakehead, Chinese and Indian carps, and tilapia are preferably cultured in earthen ponds. The pond aquaculture production is estimated to about 42%.

In Vietnam, about 4,639 fish cages are operated in four Mekong delta provinces, especially in An Giang and Dong Thap provinces, while about 17,000 ha of earthen ponds are used for fish culture there (So et al, 2010). The most commonly cultured fish species in the Lower Mekong Basin of Cambodia and Vietnam are snakehead (*Channa micropeltes* and *C. striata*), pangasiid catfish (*Pangasianodon hypophthalmus*), hybrid clarias catfish (*C. btrachus x C. gariepinus*), and giant freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture of these carnivorous and omnivorous fish species is highly dependent on inland fisheries of small-sized fish species for sourcing key dietary nutrient inputs. It is estimated that approx. 50,000 ton of freshwater small-sized fish is used for the above aquaculture development in Cambodia (So Nam et al., 2005). So Nam et al (2009) has identified approx. 200 small-sized fish species, which were used as feed for aquaculture development in the Lower Mekong basin of Cambodia and Vietnam.

The striped snakehead *Channa striata* is an obligatory air breathing fish, which can survive dry season by burrowing in bottom mud of lakes, canals and swamps as long as skin and air-breathing apparatus remain moist and subsists on the stored fat. The snakehead inhabits ponds, streams and rivers, preferring stagnant and muddy water of plains and is found mainly in swamps, but also occurs in the lowland rivers, more common in relatively deep (1-2 m), still water. The striped snakehead is a voracious carnivore feeding on fish, frogs, snakes, insects, earthworms, tadpoles and crustaceans. It is a nest-breeding species. The nest is prepared by the parent fish by clearing an area at the water surface of aquatic and emergent vegetation. It undertakes lateral migration from the Mekong mainstream or other permanent water bodies, to flooded areas during the flood season and returns to the permanent water bodies at the onset of the dry season. The striped snakehead is commonly used for processing into *prahoc*, *mam-ruot*, and *mam-ca-loc* (varieties of fish paste) in Cambodia and Vietnam. It is very economic important on both cultures and captures throughout southern and southeastern Asia. The maximal total length published is 100 cm or maximal weight 3,000 g, but commonly found 60 cm (Vidthayanon 2002; Sokheng et al., 1999; Menon, 1999).

The government of Cambodia put a ban on snakehead farming in May 2005 and the reasons for this was the potential negative impacts on wild fish populations from wasteful snakehead seed collection and on other fish species diversity, and also potential negative effects on poor consumer groups from decreased availability of small-sized/low valued fish (So et al, 2007). After the ban on snakehead culture in Cambodia, snakeheads have illegally been imported from the neighboring countries, particularly from Vietnam, to supply high local market demands in Cambodia. Furthermore, the study showed that freshwater small-sized fish have illegally been exported to Vietnam for feeding the significantly and commercially developed snakehead aquaculture in Vietnam. The first phase study funded by AquaFish CRSP revealed that the incentives for choosing snakehead before other fish species by tens of thousands of fish farmers are strong as it generates more than 10 times higher profits than other fish species (So et al., 2009). Therefore, the ban does not only result in positive impacts on poor consumer groups from increased availability of freshwater small-sized fish in Cambodia, but also providing negative effects on livelihood of tens of thousands of snakehead farmers who depend on this livelihood for generating household income. In other words, these snakehead fish farmers have lost their important livelihoods and household income. Moreover, the ban also does not provide positive impacts on snakehead wild stocks as fishing pressure on wild snakehead using illegal and destructive fishing gears particularly electro-shockers has been increased for the recent years in order to supply local and external markets (So et al., 2009).

In Vietnam, snakehead fish have been domesticated for almost two decades in the Mekong Delta (So, 2009). Aquaculture of this domesticated snakehead fish has commonly and wisely been practiced, and recently intensified by using freshwater and marine small-sized fish as direct feed. The snakehead aquaculture production increased from 30,000 ton in 2009 (Le Xuan Sinh and Do Minh Chung, 2009) to 40,000 ton in 2010 (Le Xuan Sinh, pers. comm., 2011). As a result, environmental issue and outbreak of

fish disease are the biggest problems, which cause high fish mortality due to poor water quality, and cause decreased income of hundred thousands of snakehead farmers in the Mekong Delta in Vietnam. As intensive snakehead aquaculture has been developed, many kinds of pathogens may cause serious diseases.

Some fish farmers in Cambodia have illegally imported snakehead fingerlings or broodstocks from Vietnam to continue their livelihood activity. Bringing snakehead seed and broodstocks from Vietnam may also bring diseases into Cambodia fish farms then into natural water bodies in Cambodia. As a result, wild snakehead will be infected by diseased farmed snakehead imported from Vietnam. So development of Cambodian indigenous snakehead broodstocks by domestication breeding and weaning with formulated diets will contribute positively to socio-economic development of tens of thousands of fish farmer communities as well as protect natural aquatic ecosystems. At the same time, the development of indigenous snakeheads for aquaculture in Cambodia must be approached in a responsible manner that diminishes the chance for negative environmental, technical, and social impacts. Therefore, domestication breeding and weaning of wild snakehead in Cambodia and study of water quality as well as pathogenic agents in Vietnam is practical and necessary in order to reopen snakehead aquaculture in Cambodia and to sustain snakehead aquaculture in Vietnam. Moreover, lessons learnt from Vietnam will be carried over to Cambodia. The Minister of Ministry of Agriculture, Forestry and Fisheries of Cambodia, in his letter banning snakehead culture on September 3, 2004, clearly indicated that detailed impact assessment of snakehead culture, and domesticated snakehead seed and formulated feed for weaning and growing out snakehead fish are available, the ban will be lifted.

OBJECTIVES

The specific objectives of this investigation are as follows.

1. To domesticate breeding of wild snakehead to address the snakehead banning issue in Cambodia in order to lift the ban on snakehead culture in Cambodia;
2. To study environment impacts, fish diseases and biosecurity of snakehead farming in Vietnam; and
3. To provide recommendations for policy and best practices development of snakehead farming.

The objectives 1 and 3 will be achieved by the following studies of Part 1, while the objectives 2 as well as the objective 3 will be achieved by the studies of Part 2: snakehead fish diseases and water quality analysis, under the same investigation 09IND02UC.

RESULTS

Study 1: Semi-artificial breeding of the striped snakehead *Channa striata*

1.1 Introduction

The striped snakehead *Channa striata* is widely considered an excellent food fish in Asian countries, especially in the Lower Mekong Basin countries, including Cambodia, Laos, Thailand and Vietnam, and interest in farming this snakehead is growing. One key constraint to the culture of this species is the ban on snakehead culture in Cambodia due to lack of hatchery domestication breeding fingerlings as seed material. The collection of stocking material from the wild is not sustainable. Induced spawning may be a dependable alternative for obtaining high quality seed material. The mammalian hormone, human chorionic gonadotropin (HCG) has been used as an inducing agent for ovulation and spawning in snakeheads by colleagues at Can Tho University in Vietnam and initial results have been published in the University journal in Vietnamese language (Nguyen Van Trieu et al., 2005; Nguyen Huan and Duong Nhut Long, 2008; Bui Minh Tam et al., 2008). The success of induced breeding this species also depends on the amount of HCG (dose), number of injections and injection period (Bui Minh Tam et al., 2008). The effects between doses of the inducing agent and the injection period combinations on the spawning

performance of the recipient female have not been carefully studied. Hence, in the present study the breeding performance and larvae production of *Channa striata* was investigated at different doses of HCG, number of injections and the injection period combinations.

1.2 Materials and methods

Training and technology transfer

Before the set up of experimental designs at the Freshwater Aquaculture Research and Development Center (FARDeC), its researchers have received training on snakehead on breeding, weaning, feeding strategies and feed formulation techniques (feed formulation based on the optimal diet composition: protein, lipid, mineral, fiber and energy obtained from the AquaFish CRSP first phase Investigation 07SFT01UC, and on supplemented information from Samantary and Mohanty, 1997; Arockiaraj et al, 1999) at Aquaculture and Fisheries College of Can Tho University, Vietnam. Practical work, experimental set up and snakehead farms and feed mill visits were also provided to the trainees. After the training, researchers from Can Tho University, Vietnam visited FARDeC, Cambodia to assist the trained staff to set up breeding and weaning experimental trials, prepare formulated diets and to provide advice on feeding strategies for the striped snakehead *Channa striata*.

Broodstock collection and culture

Thirty nine male and forty five female breeders of *Channa striata* were collected from the natural water bodies of Tonle Sap Great Lake in Kampong Thom province and stocked in the 300 m^2 earthen pond at Freshwater Aquaculture Research and Development Center (FARDeC) (Figure 1). The body weight of the breeders ranged from 650 gram per fish to 800 gram per fish. Low value or small-sized fish were fed to the fish with a feeding rate of 2% of body weight of fish per day. Broodstock of snakehead was monthly sampled for checking fish maturation based on the methods of Nikolsky (1963) (Table 1; Table 2 and Table 3). Total length, standard length and body weight distribution of the striped snakehead *Channa striata* used as experimental fish were measured and the sex of each specimen recorded in Table 4.

Hormone composition

The hormone used in this study is a commercial preparation of HCG (human chorionic gonadotropin). It comes as a white, lyophilized crystalline plug containing 5,000 international units (IU) per vial. Each vial of freeze-dried HCG is supplied with 5 ml of solvent containing phosphate-buffered water. The HCG was purchased and reconstituted in 1 ml of solvent provided with the pack. That was further diluted with normal saline solution to get required concentrations of injectable HCG.

Experimental designs

Experiment 1:Three groups of females were injected with 500, 1,000 and 1,500 IU HCG per kg body weight on day 2 after the last injection of three groups of males with the same dose of 1,500 IU HCG per body weight (Figure 2; Table 5). Each group of fish comprised three females or three males. The control group comprising three females and three males received 0.05 ml of solvent. Each pair of HCG treated fish (1 female: 1male) and control fish were semi-artificially bred in a 2,000-L cement tank where aquatic plants were applied to make nests for fish to lay/scatter eggs into.

Experiment 2: Three doses 2,500, 3,000 and 3,500 IU HCG per kg body weight were administered to three groups of tested males, with the tested three groups of tested females receiving a dose of 1,000 IU HCG (Table 6). All male fish received two injections from each dose for two days, while all female fish were injected with only one injection on the second day.

Experiment 3: This Experiment was set up similar to Experiment 2, but all tested male fish received three injections of HCG for three days from each of the three doses 3,000, 3,500 and 4,000 IU HCG per kg body weight (Table 7). All female fish were injected with only one injection on the third day at a dose of 1,000 IU HCG per body weight.

Experiment 4: This Experiment was conducted to assess the potency of 3,500 IU HCG per body weight using larger number of fish than the Experiment 1, 2 and 3. Six trials were conducted, utilizing twelve male and female fish, receiving 3,500 IU HCG and 1,000 IU HCG per body weight, respectively (Table 8). Control fish were always paired with hormone-treated fish.

All experiments were conducted in twelve (2 x 1 x 1 m) cement tanks for semi-artificial breeding and twelve (1.2 x 0.8 x 0. 5 m) fiberglass aquaria for egg hatching of the striped snakehead Channa striata (Figure 3). The physico-chemical parameters of hatchery water viz. temperature, pH, dissolved oxygen and total alkalinity were 27–29 °C, 6.8–7.5, 5.6–6.9 ppm and 125–132 ppm, respectively during experimental period. Each female was selected from a pool of broodstocks based on the presence of fully-yolked oocytes, using canulating method, and each male was selected based on the presence of long genital. Each female or male was conditioned in their respective cement tank for one day before the experiment commenced.

Data collection
For the Experiments 1, 2 and 3 the following data were collected, including spawning time (hour), spawning success (%), egg quantity (eggs/kg body weight), fertilization rate (%), and hatching rate (%) at the end of each period, while number of normal larvae (no. larvae/kg female) and survival rate of fish larvae (%) were also counted and recorded in Experiment 4.

Data analysis
The above collected data which are different among treatments of the same experiments were determined by one way ANOVA, with means separated using Duncan's Multiple Range test at $p = 0.05$ using the Software Program SPSS 11.0.

1.3 Results and Discussions
Broodstock development and maturation
Four stages of gonadal development of *Channa striata* were obtained in fish sampled as shown in Table 2 and Table 3. The egg diameter varied from 0.2 – 0.71 mm in stage II to 0.96 – 1.62 mm in stage IV (Table 2). All collected fish from Tonle Sap Great Lake reared in the earthen pond at Freshwater Aquaculture Research and Development Center (FARDeC) were mature. In May 46.7% of males and 60.5% of females were mature in stage IV, and this maturation rate of males and females increased to 88.8% and 80.8%, respectively in July (Table 3), the highest values that could be considered as the best month for semi-artificial breeding of the striped snakehead *Channa striata*.

Sex ratio and length and weight distribution
Out of 84 specimens examined, 39 were males and 45 were females, giving a sex ratio of 1.0: 1.2 (Table 4). The sex ratio showed an insignificant departure from the 1:1 sex ratio ($P > 0.05$). The mean body weight of males and females was 801.6 ± 4.9 g and 768.3 ± 10.2 g, which were not significantly different, while there were significant differences in the lengths between males and females for total length and standard length (Table 4).

Experiment 1: Evaluation of HCG doses for injecting male and female striped snakehead Channa stirata on spawning performance and larval hatching during spawning induction: All females injected with 1,000 IU HCG successfully spawned and provided a significantly higher number of eggs per kg body weight than females injected with 500 IU HCG and 1,500 IU HCG per kg body weight and non treated or control females (Table 5). There were no significant differences in spawning time (number of hours) among females injected with HCG doses of 1,000 IU HCG and 1,500 IU HCG per kg body weight, while these treatments were significantly different from the treatment of females injected with 500 IU HCG per kg body weight. For the control groups, no females manifested any signs of ovulation or spawning,

probably due to the lack of environment and physiological cues that would trigger final oocyte maturation. There was no fertilization among all HCG treated and non-treated or control females in this experiment, probably due to the HCG doses provided to all male fish that would be too low, resulted in the lack of physiological conditions to trigger the release of sperm.

Experiment 2 Evaluation of HCG doses for two injections for the male striped snakehead Channa stirata on spawning performance and larval hatching during spawning inductionI: There were no significant differences in fertilization rate between males treated with 3,000 IU HCG and 3,500 IU HCG per kg body weight, while male fish treated with a HCG dose of 3,500 IU per kg body weight provided a significant higher hatching rate than male fish treated with 3,000 IU HCG per kg body weight (Table 6). No fertilization and hatching was observed when all males treated with 2,500 IU HCG per kg body weight.

Experiment 3 Evaluation of HCG doses for three injections for the male striped snakehead Channa stirata on spawning performance and larval hatching during spawning induction: All HCG treated fish successfully spawned and provided no significant differences in spawning time (Table 7). All males treated with 3,500 IU HCG per kg body weight and females treated with 1,000 per kg body weight provided a significant higher number of eggs per kg body weight, fertilization rate and hatching rate than male fish injected with 3,000 IU and 4,000 IU HCG per body weight.

Experiment 4: In this experiment, the difference in spawning success between control and hormone-treated (1,000 IU HCG per kg body weight for females and 3,500 IU HCG for males) fish groups was highly significant (Table 8). The average hatching rate was 72.6%, working fecundity (number of normal larvae per kg female) 21,124 and survival rate of fish larvae after absorbing the yolk on day 3 after hatch was approx. 72%. These larvae were used for weaning experiments (See below sections).

Therefore, the best spawning performance or success (100%), highest hatching rate (81%) and highest larval productions or survival rate (72%) for the striped snakehead *Channa striata* were obtained when fish were injected with a total dose of 4,500 IU HCG at 27–29 °C, i.e. female fish receiving only one injection at a dose of 1,000 IU HCG, and male fish receiving 3 injections at a dose of 3,500 IU HCG within a period of three days or 72 hours. Ovulation or spawning occurred within 9-10 hours.

The report of Nguyen Van Trieu et al. (2005) in Vietnamese language demonstrated that they administered 2,000 IU HCG per kg body weight to female *Channa striata* and did not report the spawning success, but observed 75% hatching rate and 74% survival rate of 3-day old larvae. However, the lack of information on the selection criteria of breeders prior to injection, it is very difficult to compare their results with our present findings. Nguyen Huan and Duong Nhut Long (2008) reported in Vietnamese language that a dose of 1,000 IU HCG is effective for induced spawning in the giant snakehead *Channa micropeltes,* while Bui Minh Tam et al. (2008) found doses of 2,000-3,000 IU HCG per kg male and a dose of 500 IU per kg female to be effective for induced spawning in the giant snakehead, which are lower than the doses of the present study. The males were injected 2-3 days before the females, which this injection method is similar to the method of the present study.

Successful spawning induction was also reported by Sahoo et al. (2007) using doses of 3,000-4,000 IU HCG per kg female at 14-17 h latency. The most effective dose observed from the present study is within the recommended hormone level for induction of ovulation or spawning in the Nile tilapia (3,500 $IU.kg^{-1}$; Garcia-Abiado et al., 1994), African catfish (4,000 $IU.kg^{-1}$; Eding et al., 1982), Japanese flounder (2,600-8,400 $IU.kg^{-1}$; Hirose et al., 1979), silver carp (2,750 $IU.kg^{-1}$; Burlakov and Khachaeva, 1983), rabbitfish (2,000 $IU.kg^{-1}$, Agson, 1991), and snapper (1,000 $IU.kg^{-1}$; Pamkhurst and Carragher, 1992), but greater in sea bream (400 $IU.kg^{-1}$; Eckstein et al., 1978; Gordon and Zohar, 1978; Zohar and Gordon, 1979), and grouper (600 $IU.kg^{-1}$; Tseng and Poon, 1983). However, the dose is significantly less than that recommended by Kuo (1975) for mullet (60,000 $IU.kg^{-1}$).

Study 2: Weaning wild striped snakehead (*Channa striata*) using formulated feed

2.1 Introduction

Aquaculture of snakeheads in Cambodia is mainly dependent on freshwater small-sized fish (FSF) for sourcing key dietary nutrient inputs (So Nam et al., 2009; So Nam et al., 2005), and feeding cost is the highest cost for the fish farmer. The recent study by So Nam et al. (2009) revealed that more than 200 FSF species, with nearly 50,000 ton (accounting more than 10% of total freshwater fisheries production in Cambodia; So Nam et al., 2005) are used for aquaculture in Cambodia. Many problems are raised among many snakehead farms. The main problems are poor quality of FSF and variable nutritional composition because of inappropriate storage. Risk of disease introduction and outbreaks, environmental pollution and high feed conversion in snakehead rearing contributed more concerns. Moreover, the growing competition between human and aquaculture usage of FSF led to increasing its price to the farmer (Le Xuan Sinh et al, 2009; So Nam et al., 2009; Rachmansyah et al., 2009; So Nam et al., 2007). One key constraint and challenge to the culture of this species is the ban on snakehead culture by the government of Cambodia due to the lack of formulated diets (So Nam et al., 2009). To address the above key issues, Tran Thhi Thu Hien and her colleagues at Can Tho University in Vietnam and University of Rhode Island, USA (Tran Thhi Thu Hien Bengtson, 2009) had successfully develop cost-effective and high-performing compounded feeds under laboratory and on-farm trial conditions that would allow less reliance on FSF and would have lower environmental impacts, the so called AquaFish CRSP Snakehead Formulated Feed (Figure 4). This study, therefore, was designed to wean wild striped snakehead (*Channa striata*) using this formulated feed in order to produce fingerlings and first generation broodstocks (i.e. F_1 breeders) at the hatchery of Freshwater Aquaculture Research and Development Center (FARDeC) in Cambodia.

2.2 Materials and methods

Training and technology transfer

The same as and please see section 1.2.

Experiment 1: Effects of weaning methods using formulated feed for the striped snakehead (Channa striata) on survival rate, dead rate and cannibalism rate

Experimental fish

Before starting the experiment, all 3-day old larvae after hatch were nursed in 1000-L fiber tanks (Figure 5) and fed with *Moina* for 7 days, then fed with *Moina* and freshwater small-sized fish (FSF) for 10 days, 20 days and 30 days to obtain 20 day-old fish, 30 day-old fish and 40 day-old fish, respectively. Replacement of *Moina* by FSF was applied gradually at a rate 10%.day^{-1} until 100% of *Moina* was substituted by FSF.

Experimental design

We tested three ages of fish to begin weaning with freshwater small-sized fish gradually replaced by 45% crude protein formulated feed (Table 9; Tran Thi Thu Hien and Bengtson, 2009) for 30 days: 20 day-old (20-dof), 30 day-old (30-dof) and 40 day-old (40-dof) fish (Table 10). For 20-dof treatments, the weaning procedure consisted of 10% of freshwater small-sized fish replaced daily, every two days and every three days by formulated feed until fish were fed exclusively on formulated feed. Similarly, for the 30-dof and 40-dof treatments, freshwater small-sized fish biomass was replaced by formulated diet at a rate of 10% per day, per two days and per three day for each treatment. Control treatment, 20-dof fish were fed with *Moina* replaced by freshwater small-size fish 20% per day within five consecutive days and then fed with freshwater small-sized fish until the end of the experiment (Table 10).

Fish were randomly assigned in 30 (2 x 1 x 1 m) hapas placed in 2,000 m^2 ponds, i.e. 10 treatments and 3 replicates per treatment, at a stocking density of 200 fish/hapa or 100 fish/m^2. The fish were fed to

satiation by hand twice daily at 09:00 h and 16:00. Dead fish and cannibalism were weekly recorded (Table 11). Water temperature, pH and dissolved oxygen also recorded weekly, ranging from 28.2 – 29.5; 5.4 – 6.7; and 5.7 – 6.9 ppm, respectively. Differences among treatments were determined by one way ANOVA with means separated using Duncan's Multiple Range test at p = 0.05 using SPSS 11.0.

Experiment 2: On-station trial on growth-out of the striped snakehead Channa striata fed with freshwater small-sized fish vs. formulated feed
Experimental fish and designs
Before starting the experiments, all the 30 day-old fish were reared in 10 (5 x 3 x 1.5 m) hapas, placed in 2,000 m^2 ponds, with a stocking density of 100 fish/m^2 and fed with freshwater small-sized fish combined with 45% crude protein formulated feed (Table 9) for 30 days at the hatchery of Freshwater Aquaculture Research and Development Center (FARDeC), Cambodia. Replacement of freshwater small-sized fish by formulated feed was applied gradually at a rate 10% every three days until 100% of freshwater small-sized fish was substituted by pellet feed, then the fish were fed with formulated or pellet feed till day 30^{th} (i.e. the age of the fish was 60 days old).

Experiment 2 was set up by using the above 60 day-old fish of the striped snakehead *Channa striata* to evaluate effects of freshwater small-sized fish and 40% crude protein pellet feed on growth performance, survival rate, feed intake, feed conversion ratio, and abnormal rate, and to develop first generation of broodstock (F_1) for further domestication breeding. Fish were randomly stocked in six (5 x 3 x 1.5 m) hapas placed in 2,000 m^2 ponds (Figure 6) at a stocking density of 750 fish/hapa, i.e. being three hapas for fish fed with freshwater small-sized fish and three hapas for fish fed with pellet feed. The fish were fed to satiation by hand twice daily at 09:00 h and 16:00. Total fish weight in each hapa was determined every month and dead fish were recorded and weighed for calculating feed conversion ratio (FCR). After feeding, the remaining feed was weighed daily. Water temperature, pH and dissolved oxygen were measured biweekly, ranging from 27.9 - 30.5 ^oC; 5.2 – 6.7; and 5.4 –7.1 ppm, respectively. The experiment lasted 10 months. Collected data, which were different among treatments, were determined by one way ANOVA with means separated using Duncan's Multiple Range test at p = 0.05 using SPSS 11.0.

2.3 Results and Discussions
Experiment 1: Effects of weaning methods using formulated feed for the striped snakehead (Channa striata) on survival rate, dead rate and cannibalism rate: Survival rates of fish in treatments 20-dof-1, 20-dof-2 and 20-dof-3 were significantly lower than those of all other treatments, while these treatments had significantly higher cannibalism than those of all other treatments (Table 11). This reveals that the age of these fish is too young to accept formulated feed resulting in significant higher cannibalism (18 – 26%). Survival rate and cannibalism rate of fish in treatment 30-dof-3 was 75% and 12%, respectively, and were not significantly different from those of fish in the control treatment (fish fed with *Moina* and freshwater small-sized fish) and in treatment 40-dof-3, but significantly higher than those of fish in treatments 30-dof-2, 30-dof-2, 20-dof-1, 20-dof-2 and 20-dof-3. Therefore, the fish aging 30 days old can gradually and successful accept formulated feed in replacement of small-sized fish in the rate of 10% every three days.

Experiment 2: On-station trial on growth-out of the striped snakehead Channa striata fed with freshwater small-sized fish vs. formulated feed: The final weight of fish fed with freshwater small-sized fish (468 g) significantly higher than that of fish fed with formulated or pellet feed (314 g) (Table 12), but this figure is lower than the striped snakehead fed with small-sized fish in the Mekong delta of Vietnam (0.5 -1.1 kg/fish; So Nam, 2009; Le Xuan Sinh and Pomeroy, 2009). Similarly the final weight of the snakehead fish fed with pellet feed in this study is lower than the fish fed with pellet feed in Dong Thap and An Giang provinces (467 - 726 g/fish; Tran Thi Thanh Hien and Bengtson, 2011). This higher growth rate in both cases reflects the longer and successful domestication captive breeding of striped snakehead in the Mekong Delta of Vietnam. However the survival rate of fish in both treatments was not significantly

different after a growth-out period of 10 months, although a slightly higher survival rate was found in treatment of fish fed with freshwater small-sized fish (60%) than that (56%) in treatment of fish fed with pellet feed. These survival rates are similar to the rates of striped snakehead fed with small-sized fish (45-60%; So Nam, 2009; Le Xuan Sinh and Pomeroy, 2009) and to the fish fed with pellet feed (56-73%; Tran Thi Thanh Hien and Bengtson, 2011) in the Mekong Delta of Vietnam. The high mortality rate of striped snakehead fed with small-sized fish found in the Mekong Delta is caused by parasite infected diseases (40-50%; Pham Minh Duc et al., 2011; Le Xuan Sinh and Pomeroy, 2009).

Table 13 shows that FI and FCR were significantly higher in treatment of fish fed with freshwater small-sized fish (3.9 g.fish-1.day^{-1} and 4.2, respectively) than in treatment of fish fed with pellet feed (1.25 g.fish-1.day^{-1} and 1.68, respectively), but no significant differences were seen in PER. It is likely that the significant reductions in FI and FCR were due to different moisture levels in the diets that the fish received, since the moisture of freshwater small-sized fish is 72.7 %, whereas formulated feed is 9.38% (Tran Thi Thu Hien and Bengtson, 2009). The treatment of fish fed with pellet feed (17.4%) had a significantly higher abnormal rate than the treatment of fish fed with freshwater small-sized fish. The FCR of striped snakehead fed with small-sized fish in the Mekong Delta of Vietnam (3.7 - 4.5; Tran Thi Thanh Hien and Bengtson, 2011; Le Xuan Sinh and Pomeroy, 2009) is similar to the FCR in this study, while the FCR of fish fed with pellet feed in this study is higher than the that of fish fed with pellet feed in the Mekong Delta of Vietnam (1.3 - 1.6).

In this study, the poorer growth rate of fish fed with pellet feed compared with fish fed with freshwater small-sized fish, probably due to that the experimental fish used originate from the wild breeders, which have been collected from Tonle Sap Great Lake and first time brought into the hatchery of FARDeC for induced spawning. In order words, the experimental fish used in this study are wild fish, while the experimental fish of the same species used in the study of Tran Thi Thu Hien and Bengtson (2009) at Can Tho University, Vietnam have been successfully domestication induced spawning for nearly two decades at hatcheries in Vietnam. Similarly, the study conducted by colleagues at Can Tho University showed that the growth rate and survival rate of the giant snakehead *Channa micropeltes* fed with formulated feed is significantly lower or poorer than the fish fed with small-sized or low value fish in Vietnam (Tran Thi Thu Hien, pers. comm., 2011), probably due to that the giant snakehead have been just recently domestication breeding for nearly 5 years.

The striped snakehead *Channa striata* is an obligatory air breathing and a carnivorous fish species. In this study, although they were weaned from freshwater small-sized fish to formulated feed, live food is still their favorite feed. The diet containing no freshwater small-sized fish reduced attraction of fish to feed (Tran Thi Thu Hien and Bengtson, 2009). Utilization of commercial pellet feed nowadays is more popular, especially for carnivorous fish in order to reduce the dependence on small-sized fish or low value fish, feeding cost and environmental impact. Several studies on replacing of small-sized fish or low value fish by formulated feed in several species achieved better growth rate and more profit, such as this species (Tran Thi Thu Hien and Bengtson, 2009); tiger grouper, *Epinephelus fuscoguttatus* (Rachmansyah et al., 2009); Japanese sea bass, *Lateolabrax japonicus* and red drum, *Sciaenops ocellata* (Cremer et al., 2001); Sea bass, *Lates calcarifer* (Aquacop et al., 1989). Cremer et al. (2001) replaced small-sized fish or low value fish with formulated diets in cage culture of red drum (*Sciaenops ocellata*) (172g/fish in initial weight) and Japanese sea bass *Lateolabrax japonicus*) (74g/fish) and concluded that fish consuming formulated diet (43% crude protein, 12% lipid) with 35% soybean meal showed better growth and less feeding cost than fish fed with small-sized fish.

In this study, *Channa striata* showed a high cannibalism in pellet feed treatments. Lower survival rate may be related to snakehead behavior. Conversely, Hung et al. (1999) reported that *Pangasius bocourti* had a low cannibalism even in the artificial feeding treatment. Simply providing formulated feeds led to cannibalism in *Channa striata* in a previous study (Qin et al., 1996). In fishes, cannibalism is usually

associated with heterogeneous size variation, lack of food, high density, lack of refuge area and light condition. Among these variables, size variation and unsuitable food are considered the primary causes of cannibalism (Tran Thi Thu Hien and Bengtson, 2009).

CONCLUSIONS

The present investigation demonstrated that the wild striped snakehead *Channa striata broodstock*s can successfully be developed, mature and semi-artificially induced spawning using HCG at doses of 1,000 $IU.kg^{-1}$ for female fish and 3,500 $IU.kg^{-1}$ for male fish at the hatchery of Freshwater Aquaculture Research and Development Center (FARDeC), Cambodia. The male fish receive 2-3 injections within 2-3 days before the female fish, which is received only 1 injection. With this optimal HCG doses, the spawning success is 100%; spawning time after the last injection of female and male fish is 9 hours; number of eggs spawned per kg female is 32,000; the fertilization rate is 87%; hatching rate is 73%; and the larval production and survival rate is 21,000 larvae per kg female and 72%, respectively.

The striped snakehead *Channa straita* aging 30 days old after hatch can gradually and successful accept formulated feed in replacement of small-sized fish in the rate of 10% every three days for a period of 30 days of feeding, and then be successfully grown out with a complete 40% crude protein pellet feed for a period of ten months to achieve a final weight of 314 $g.fish^{-1}$, a survival rate of 56%, and a FCR of 1.68. The F_1 broodstocks which can accept formulated or pellet feed are available for future domestication breeding and weaning at FARDeC, Cambodia. This has very important implications for protecting freshwater small-sized fish, which are usually fed to snakehead.

RECOMMENDATIONS

The following recommendations should be carefully considered for policy and action plan development in order to lift the ban on snakehead and achieve sustainable development snakehead aquaculture in Cambodia:

- To collect from different natural water bodies over the country and develop sufficient numbers of *Channa striata* broodstcoks at hatcheries in Cambodia in order to conduct R & D on breeding and weaning techniques to produce high quality fish seed for sustainable snakehead aquaculture development;
- To biologically characterize the snakehead *Channa striata* from different populations within Cambodia freshwater bodies (i.e. Tonle Sap Great Lake, upper and lower stretch of Mekong River and Bassac River, their associated floodplains) for determining good or favorable traits for aquaculture development;
- To assess genetic diversity and populations of snakehead collected from different locations within Cambodia for maintaining diversity of wild stocks and overall conservation of this species, and for enhancing the diversity of snakehead breeders when conducting domestication/breeding program for this fish;
- To domesticate breeding of wild snakehead to address the snakehead banning issue in Cambodia in order to lift the ban on snakehead culture in Cambodia;
- To develop practical formulated diets for broodstock, nursery and grow-out culture of snakehead to replace small-sized fish from captured fisheries;
- To evaluate the growth performance of snakehead in different culture systems by using practical formulated diets; and
- Provide extension services to snakehead farmers regarding technologies of snakehead breeding, weaning and growth-out using formulated diets; and

- To encourage the involvement of public and private sectors and development partners to invest on the value chain of snakehead aquaculture development, especially the private sector to formulate and improve commercially manufactured feed for snakehead aquaculture; it can be better integrated into local economy with less import of ingredient, and be market at a lower price.

ACKNOWLEDGEMENTS

This study was funded by AquaFish CRSP. We thank the Cambodia's Fisheries Administration and Tonle Sap Fisheries Conservation Center for field support and collaboration. We thank colleagues, field researchers and graduate and under graduate students of Inland Fisheries Research and Development Institute (IFReDI) in Phnom Penh for conducting snakehead breeding and weaning experiments at Freshwater Aquaculture Research and Development Center (FARDeC), and College of Aquaculture and Fisheries Can Tho University in Vietnam for providing trainings on snakehead breeding and weaning and their kind cooperation.

REFERENCES

Agson FC (1991). Induced spawning of rabbitfish *Signus guttatus* (Bloch) using human chorionic gonadotrophin (HCG). Aquaculture 95: 133-137.

Aquacop, G. Cuzon, R. Chou and J. Fuchs, 1989. Nutrition of the Seabass (Lates calcarifer). Advances in tropical aquaculture Tahiti. 9: 757-763.

Arockiaraj, A.J., M. Muruganandam, K. Marimuthu and M.A. Haniffa. 1999. Utilization of carbohydrates as a dietary energy source by striped murrel, *Channa striatus* (Bloch) fingerlings. Acta Zool.Taiwan 10: 103-111.

Bui Minh Tam, Nguyen Thanh Phuong and Duong Nhut Long (2008). Effects of HCG dosages and injection methods on semi-artificial propagation of giant snakehead (*Channa micropeltes*). Aquaculture and Fisheries College, Can Tho University, Vietnam (Tap chi Khao hoc 2008 (2): 76:81)

Cremer, M.C., Z. Jian and H.P. Lan, 2001. Cage Production of Japanese Sea bass Weaned From Trash Fish to Extruded Feed at Sub-Market Size. Results of ASA/China Feeding Trial 35-01-128.

Eckstein BM, Abraham BM and Zohar Y (1978). Production of steroid hormones by male and female gonads of *Sparus aurata* (Teleost, Sparidae). Comparative Biochemistry and Physiology 60B:93-97

Eding EH, Jansen JAL, Klein GHJ and Ritcher CJJ (1982). Effects of human chorionic gonadotrophin (HCG) on maturation and ovulation of oocytes in the catfish *Clarias lazera* (C &V). Proceedings of the internation symposium of rpeorduction and physiological fisheries, Pudoc, Wageningen, the Netherlands: 195.

Gacia-Abiado MAR, Pascual LP and Mair GC (1994). Use of Human Chorionic Gonadotropin (hCG) for Induced Spawning in Tilapia Under Laboratory Conditions. *Asian Fisheries Science* 7: 225-231

Gordin H and Zohar Y (1978). Induce spawning of *Sparus aurata* L. by means of hormonal treatments. Annals de Biologie Biophyisque 18 (4):985-990.

Hirose K, Machida Y and Donaldson EM (1979). Induced ovulation of Japanese flounder (*Limauda yokohamae*) with human chorionic gonadotrophin (HCG) and salmon gonadotrophin, with special references to changes in quality of eegs retained in the ovarian cavity after ovulation. Bulletin-Japanese Society of Scientific Fisheries 45 (1): 31-36

Hung, L.T., B.M. Tam, P.Cacot and J.Lazard, 1999. Larval rearing of the Mekong catfish, Pangasius bocourti (Pangasidae, Siluroidei): Substitution of Artemia nauplii with live and artificial feed. Aquatic Living Resource, 12(3):229-232

Kuo CM (1975). Recent progress on the control of ovarian developmentand induced spawning of the grey mullet (*Mugil cephalus* L.). Aquaculture 5: 19-29.

Kurlakov AB and Khachaeva EV (1983). Oocyte maturation in silver carp, *Hypopthalichthys molitirx* (Cyprinidae) and in vitro effect of estrogens, androgens and gestogens in relation to in vivo sensitivity to human chorionic gonadotrophin (HCG). Journal of Ichthyology 23 94): 124-132.

Lê Xuân Sinh và Đỗ Minh Chung. 2009. Hiện trạng và những thách thức cho nghề nuôi cá lóc (Channa micropeltes và Channa striatus) ở ĐBSCL. Báo cáo trình bày tại Hội thảo kết thúc giai đoạn 1 – Dự án Cá Tạp - Khoa Thủy sản - ĐHCT, 8-12/20

Le Xuan Sinh and Pomeroy (2009). Competition And Impacts Between Uses Of Low Value Fish For Aquaculture Feed Versus Uses For Human Food In The Lower Mekong Basin Of Cambodia & Vietnam. Final Technical Report of AquaFish CRSP USAID Grant No.: EPP-A-00-06-00012-00. Can Tho University, Vietnam and University of Connecticut, USA.

Menon, A.G.K. 1999 Check list - fresh water fishes of India. Rec. Zool. Surv. India, Misc. Publ., Occas. Pap. No. 175, 366 p.

Nguyen Huan and Duong Nhut Long (2008). The hatchery status and technical aspects for snakehead spawning (*Channa micropeltes*). Aquaculture and Fisheries College, Can Tho University, Vietnam (Tap chi Khao hoc 2008 (2): 20:28)

Nguyen Van Trieu, Duong Nhut Long, Le Son Trang (2005). Seed production technology of Snakehead Fish (*Channa striatus* Bloch). Aquaculture and Fisheries College, Can Tho University, Vietnam

Nikolsky GV (1963). The ecology of fishes. Academic Press. London and New York. p. 352.

Pankhurst NW and CarragherJF (1992). Oocyte maturation and changes in plasma steroid levels in snapper *Pagrus (=Chrysophrys) auratus* (Sparidae) following treatment with human chorionic gonadotrophin (HCG). Aquaculture 101:337-347.

Qin, J., A.W.F., 1996. Size and feed dependent cannibalism with juvenile snakehead Channa striatus. Aquculture 4, 313-320.

Rachmansyah, U., Palinggi, N.N. and Williams, K., 2009. Formulated feed for tiger grouper grow-out, AquacultureAsia Magazine, pp. 30-35.

S K Sahoo, S S Giri, S Chandra, A K Sahu (2007). Spawning performance and egg quality of Asian catfish *Clarias batrachus* (Linn .) at various doses of human chorionic gonadotropin (HCG) injection and latency periods during spawning induction. Aquaculture, 266: 289-292

Samantaray, K. and S.S. Mohanty (1997). Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata*. Aquaculture 156: 245-253.

So Nam & Touch Bunthang (2011). Fisheries Resources in Cambodia: Implications for Food Security, Human Nutrition and Conversation. International Conference on Asian Food Security (ICAFS2011) -- "Feeding Asia in the 21st Century: Building Urban – Rural Alliances", 10-12 August 2011 at the Grand Copthorne Hotel, Singapore.

So Nam (2009). Snakehead culture in the Mekong Delta of Vietnam. Trip Report of AquaFish CRSP USAID Grant No.: EPP-A-00-06-00012-00. Inland Fisheries Research and Development Institute, Phnom Penh.

So Nam and Haing L (2007). Assessment of Freshwater Fish Seed Resources in Cambodia. In: MG Bondad-Reantaso (ed.): FAO Fisheries Technical Paper No. 501: Assessment of Freshwater fish seed resources for sustainable aquaculture pp. 145-170.

So Nam and Srun Lim Song (2011). Fisheries Management and Development in Tonle Sap Great Lake, Cambodia. Paper presented at Consultation Workshop On Development Trends In Fisheries And Aquaculture In Asian Lakes And Reservoirs, September, 2011 at Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, PR China.

So Nam, Eng Tong, Souen Norng and Kent Hortle (2005). Use of freshwater low value fish for aquaculture development in the Cambodia's Mekong basin. Consultancy report for Mekong River Commission – Assessment of Mekong Capture Fisheries Project. Inland Fisheries Research and Development Institute, Department of Fisheries, Phnom Penh, Cambodia.

So Nam, Leng Sy Vann, Prum Somany, Le Xuan Sinh, and Pomeroy Robert (2009). Assessment Of Diversity And Bioecological Characteristics Of Low Value/Small-Sized Fish In The Lower Mekong River Basin Of Cambodia And Vietnam. Final Technical Report of AquaFish CRSP USAID Grant No.: EPP-A-00-06-00012-00. Inland Fisheries Research Development Institute, Phnom Penh and University of Connecticut, USA.

Sokheng, C., C.K. Chhea, S. Viravong, K. Bouakhamvongsa, U. Suntornratana, N. Yoorong, N.T. Tung, T.Q. Bao, A.F. Poulsen and J.V. Jørgensen 1999 Fish migrations and spawning habits in the Mekong mainstream: a survey using local knowledge (basin-wide). Assessment of Mekong fisheries: Fish Migrations and Spawning and the Impact of Water Management Project (AMFC). AMFP Report 2/99. Vientiane, Lao, P.D.R.

Tran Thi Thanh Hien and Bengtson D (2011). Alternative Feeds for Freshwater Aquaculture Species. Final Technical Report of AquaFish CRSP USAID Grant No.: EPP-A-00-06-00012-00. Can Though University, Vietnam and University of Rhode Island, USA.

Tran Thi Thanh Hien and Bengtson D (2009). Alternative Feeds for Freshwater Aquaculture Species. Final Technical Report of USAID Grant No.: EPP-A-00-06-00012-00. Can Though University, Vietnam and University of Rhode Island, USA.

Tseng WY and Poon CT (1983). Hybridization of Epinephalus species. Aquaculture 34: 177-182.

Vidthayanon, C. 2002 Peat swamp fishes of Thailand. Office of Environmental Policy and Planning, Bangkok, Thailand, 136

Zohar Y and Gordin H (1979). Spawning kenetics in gillhead seabream Sparus aurata L., after low doses of human chorionic gonadotrophin (HCG). Journal of Fish Biology 15: 665:670.

LIST OF FIGURES

Figure 1. AquaFish CRSP earthen pond of wild striped snakehead breeders (*Channa striata*) collected from the Tonle Sap Great Lake at the FARDeC hatchery, Cambodia

Figure 2 Conditioning and HCG injection of tested male and female breeders of the striped snakehead *Channa striata*

Figure 3 Breeding cement tanks (2 x 1 x 1 m), with aquatic plants for the striped snakehead *Channa striata* scatter their eggs into

Figure 4 AquaFish CRSP snakehead formulated feed by College of Aquaculture and Fisheries, Can Tho University, Vietnam

Figure 5 Fiber tanks used for nursing larvae of the striped snakehead *Channa striata* at FARDeC hatchery, Cambodia

Figure 6 Hapas used for growing out striped snakehead (*Channa striata*) fed with formulated feed at FARDeC, Cambodia

LIST OF TABLES

Table 1 Stages of gonad development classified by Nikolsky (1963)

Variable	Stage	Gonad development
Immaturity	I	Young individuals which have not yet engaged in reproduction. Gonads of very small size.
Resting	II	Sexual products have not yet begun to develop. Gonads of very small eggs not distinguished to the naked eyes.
Maturation	III	Eggs distinguishable to the naked eyes. A very rapid increase in weight of the gonad in progress, testes changes from transparent to a pale rose color.
Maturity	IV	Sexual product, ripe gonads have achieved their maximum weights but the sexual products are not still extruded when light pressure is applied.
Reproduction	V	Sexual products are extruded in responses to very light pressure on the belly. Weight of the gonads decreases rapidly from the start of spawning to its completion.
Spent	VI	The sexual products have been discharged, genital aperture is inflamed, gonads have appearance of a deflated sac and ovaries usually containing a few left over eggs and the testes contain residual sperm.

Table 2 Four stages of gonad development of the striped snakehead *Channa striata*

Variable	Stage	Egg diameter (mm)	Gonad development
Immaturity	I	Slightly granular	Slightly granular, transparent with clear nucleus.
Resting	II	0.20 – 0.71	Clearly granular, not visible nucleus, some spherical shape and opaque.
Maturation	III	0.72 – 0.95	Spherical, yellow in color with oil globules and large ova translucent.
Maturity	IV	0.96 – 1.62	Spherical, golden yellow, translucent with oil globules.

Table 3 Monthly maturation rate of gonad development stages of the male and female striped snakehead *Channa striata*

Month	Stage I (%)		Stage II (%)		Stage III (%)		Stage IV (%)		Stage V (%)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
March	3.4	7.9	20.2	15.3	76.4	53.3	0.0	23.5		
April		2.6	5.5	9.7	81.2	42.9	13.3	44.8		
May			2.1	3.6	51.2	35.9	46.7	60.5		
June					35.7	27.5	64.3	72.5		
July					11.2	19.2	88.8	80.8		
August							60.5	63.5	39.5	36.5

Table 4 Total length, standard length and body weight distribution of the striped snakehead *Channa striata* used as experimental fish

Parameter	Sex	Number	Range	Mean
Total length (cm)	Male	39	45.9 – 55.3	46.8 ± 3.2[a]
	Female	45	41.4 – 60.1	42.6 ± 2.7[b]
Standard length (cm)	Male	39	42.3 – 49.7	43.2 ± 2.5[a]
	Female	45	38.6 – 52.5	39.8 ± 1.3[b]
Body weight (g)	Male	39	692.4 – 855.4	801.6 ± 4.9[a]
	Female	45	625.7 – 785.7	768.3 ± 10.2[a]

Table 5 Evaluation of HCG doses for injecting male and female striped snakehead *Channa stirata* on spawning performance and larval hatching during spawning induction

Description	Dose of HCG (IU) per kg body weight				
	Control	Male		Female	
	0	1,500	500	1,000	1,500
Day 1	0	500	0	0	0
Day 2	0	1000	500	1000	1500
Spawning time (h)[1]	-		15.8 ± 2.2[a]	10.5 ± 0.8[b]	8.6 ± 2.6[b]
Spawning success (%)[2]	0		33.3	100.0	66.7
Egg quantity (eggs/kg)	0		5,198 ± 688[a]	22,115 ± 1019[b]	15,939 ± 1251[c]
Fertilization rate (%)	0		0	0	0
Hatching rate (%)	0		0	0	0

Data are means of three observations ± SE. Means in the same row with different superscripts are significantly different (P<0.05).

[1] *Spawning time refers to number of hours post injection when female fish lay eggs.*

[2] *Spawning success refers to number of ovulated females as a percentage of treated females.*

Table 6 Evaluation of HCG doses for two injections for the male striped snakehead *Channa stirata* on spawning performance and larval hatching during spawning induction

Description	Dose of HCG (IU) per kg body weight			
	Female		Male	
	1,000	2,500	3,000	3,500
Day 1	0	500	1,000	1,500
Day 2	1,000	1,000	2000	2,000
Spawning time (h)[1]		14.9 ± 1.7[a]	11.1 ± 0.9[b]	9.3 ± 0.7[b]
Spawning success (%)[2]		66.7	66.7	100.0
Egg quantity (eggs/kg)		10,198 ± 1,581[a]	27,115 ± 2,917[b]	32,792 ± 1,251[b]
Fertilization rate (%)		0[a]	79.5 ± 2.1[b]	87.2 ± 3.3[b]
Hatching rate (%)		0[a]	71.9 ± 1.7[b]	81.21 ± 4.4[c]

Data are means of three observations ± SE. Means in the same row with different superscripts are significantly different (P<0.05).

[1] *Spawning time refers to number of hours post injection when female fish lay eggs.*

[2] *Spawning success refers to number of ovulated females as a percentage of treated females.*

Table 7 Evaluation of HCG doses for three injections for the male striped snakehead *Channa stirata* on spawning performance and larval hatching during spawning induction

Description	Dose of HCG (IU) per kg body weight			
	Female		Male	
	1,000	3,000	3,500	4,000
Day 1	0	500	500	500
Day 2	0	1,000	1,000	1,000
Day 3	1,000	1500	2,000	2,500
Spawning time (h)[1]		12.3 ± 0.4[a]	10.1 ± 0.9[a]	8.3 ± 2.7[a]
Spawning success (%)[2]		100.0	100.0	100.0
Egg quantity (eggs/kg)		22,835 ± 2,407 [a]	33,088 ± 2,971[b]	17,985 ± 1,911[c]
Fertilization rate (%)		81.2 ± 1.9[a]	93.6 ± 2.2[b]	49.2 ± 5.3[c]
Hatching rate (%)		65.4 ± 2.7[a]	79.8 ± 3.7[b]	40.9 ± 4.4[c]

Data are means of three observations ± SE. Means in the same row with different superscripts are significantly different (P<0.05).

[1] *Spawning time refers to number of hours post injection when female fish lay eggs.*

[2] *Spawning success refers to number of ovulated females as a percentage of treated females.*

Table 8 Number of ovulated and spawning females, egg quantity, fertilization rate, hatching rate and larvae production following with the injection of HCG potency of 3,500 IU per kg body weight

Description	Dose of HCG (IU) per kg body weight		
	Male and female	Female	Male
	Control	1,000	3,500
Day 1	0	0	500
Day 2	0	0	1,000
Day 3	0	1,000	2,000
Spawning time (h)[1]	-		9.2 ± 0.6
Spawning success (%)[2]	0		100.0
Egg quantity (eggs/kg)	0		31,935 ± 1,874
Fertilization rate (%)	0		86.8 ± 4.1
Hatching rate (%)	0		72.6 ± 3.3
Number of normal larvae per kg female	0		21,124 ± 1,025
Survival rate of fish larvae (%)[3]	0		71.8 ± 7.2

[1] *Spawning time refers to number of hours post injection when female fish lay eggs.*

[2] *Spawning success refers to number of ovulated females as a percentage of treated females.*

[3] *Survival rate of fish larvae refers to number of fish larvae as percentage of hatched larvae after absorbing the yolk on day 3 after hatch.*

Table 9 The formulation of formulated feed (45% crude protein and 4.5 gross energy per g) for the striped snakehead *Channa striata* developed by Tran Thi Thu Hien and Bengtson (2009)

Ingredient	Ratio (%)
Fish meal	32.7
Soy bean meal	31.8
Rice bran	20.0
Cassava meal	7.12
Vitamin	1.00
Mineral	1.00
Fish oil	3.32
Binder	0.29
Lysine	0.44
Methionine	0.28
Threonine	0.40
Fish solution attractant	1.5
Phytase	0.02
Total	100.00

Table 10 Experimental designs for replacing freshwater small-sized fish by 10% formulated feed fed to three ages of experimental fish for every day, every two days and every three days: 20 days, 30 days and 40 days old fish (20-dof-1, 2, 3; 30-dof-1, 2, 3 and 40-dof-1, 2, 3)

Treatment	Feed	Fish age (day)	Replacement ratio
Control	Moina + FSF	20	20% FSF every day
20-dof-1	FSF + formulated feed	20	10% formulated feed per day
20-dof-2	FSF + formulated feed	20	10% formulated feed every two days
20-dof-3	FSF + formulated feed	20	10% formulated feed every three days
30-dof-1	FSF + formulated feed	30	10% formulated feed per day
30-dof-2	FSF + formulated feed	30	10% formulated feed every two days
30-dof-3	FSF + formulated feed	30	10% formulated feed every three days
40-dof-1	FSF + formulated feed	40	10% formulated feed per day
40-dof-2	FSF + formulated feed	40	10% formulated feed every two days
40-dof-3	FSF + formulated feed	40	10% formulated feed every three days

FSF: Freshwater small-sized fish

Table 11 Survival rate, dead rate and cannibalism rate of three different ages of striped snakehead (*Channa striata*) gradually fed with formulated feed

Treatment	Survival rate (%)	Dead rate (%)	Cannibalism rate (%)
Control	85.6 ± 4.2^{a}	9.6 ± 3.2^{a}	4.8 ± 4.5^{a}
20-dof-1	36.2 ± 6.3^{bc}	45.9 ± 6.8^{b}	17.9 ± 10.1^{bc}
20-dof-2	38.9 ± 3.7^{c}	40.4 ± 8.5^{bc}	20.7 ± 7.6^{bc}
20-dof-3	41.1 ± 15.3^{c}	33.1 ± 11.2^{c}	25.8 ± 5.1^{c}
30-dof-1	68.9 ± 6.9^{d}	21.7 ± 6.3^{d}	9.4 ± 15.3^{d}
30-dof-2	62.2 ± 9.6^{d}	17.4 ± 10.2^{d}	20.4 ± 3.3^{d}
30-dof-3	74.6 ± 7.1^{ae}	13.3 ± 2.1^{ae}	12.1 ± 2.9^{ae}
40-dof-1	63.9 ± 9.4^{e}	29.7 ± 15.5^{be}	6.4 ± 5.1^{e}
40-dof-2	80.4 ± 5.4^{ae}	14.7 ± 13.9^{ae}	4.9 ± 4.4^{e}
40-dof-3	82.5 ± 2.3^{ae}	8.1 ± 2.8^{ae}	9.4 ± 3.9^{ae}

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different ($P<0.05$).

Table 12 Total body weight gain (g.fish^{-1}), daily weight gain (g.fish-1.day^{-1}) and survival rate (%) of *Channa striata* in treatments where freshwater small-sized fish and formulated feed applied

Feed	Initial weight	Final weight	Total weight gain	Daily weight gain	Survival rate
Freshwater small sized fish	9.8 ± 0.05[a]	467.9 ± 92.8[a]	458.1 ± 92.6[a]	1.5 ± 0.3[a]	60.2 ± 8.1[a]
Pellet feed	9.7 ± 0.17[a]	313.5 ± 110.4b	303.8 ± 110.2[b]	1.0 ± 0.4[b]	55.9 ± 12.3[a]

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different (P<0.05).
Total gain weight = Final weight – Initial weight
Daily gain weight = (final weight – initial weight)/experiment time
Survival rate = (numbers of fish at the end of experiment / numbers of initial fish) x 100

Table 13 Feed intake (g.fish-1.day^{-1}), feed conversion ratio, protein efficiency ratio (protein gain-1) of the striped *Channa striata* in treatments where freshwater small-sized fish and pellet feed applied (% of moisture matter basis)

Feed	FI	FCR	PER	Abnormal rate (%)
Freshwater small sized fish	3.9 ± 0.18[a]	4.2 ± 0.07[a]	2.11 ± 0.03[a]	6.3 ± 1.8[a]
Pellet feed	1.25 ± 0.11[b]	1.68 ± 0.05[b]	1.81 ± 0.07[b]	17.4 ± 3.7[b]

Data are means of three observations ± SE. Means in the same column with different superscripts are significantly different (P<0.05).
FI (Feed Intake) = (Feed intake/no fish)/ No days
FCR (Feed Conversion Ratio) = Feed intake / Weight gain
PER (Protein Efficiency Ratio) = (Final body weight – Initial body weight) / Protein intake
Abnormal rate = (number of abnormal fish/total number of survived fish) x 100

Part 2: Striped Snakehead Fish Diseases and Water Quality Analysis

Pham Minh Duc and Tran Thi Thanh Hien
Can Tho University
Can Tho, Vietnam

So Nam
Inland Fisheries Research and Development Institute
Phnom Penh, Cambodia

Robert S. Pomeroy
University of Connecticut – Avery Point
Groton, Connecticut, USA

INTRODUCTION

In the recent years, aquaculture played an economically important strength in Mekong Delta. Catfish, snakehead fish and freshwater prawn contributed in increasing of freshwater aquaculture production. Of which, snakehead was an important species in culture in Mekong Delta (Nguyen Van Thuong, 2004). There are some popular culture systems, including of pond culture, hapa culture, cage culture and nylon tank (Le Xuan Sinh and Do Minh Chung, 2010). Collecting information of provinces in Mekong Delta showed that the snakehead production in region was about 30,000 tons, of which *Channa micropelte* was 7,500 tons (2009). However, the farmers in Mekong Delta attended to small scale systems and spontaneous systems (Le Xuan Sinh and Do Minh Chung, 2010). There are 4 species of *Chanidae* in Mekong Delta, comprising of *Channa gachua*, *Channa lucius*, *Channa striata*, *Channa micropelte*. *Channa striata* and *Channa micropelte* are main cultured species in Mekong Delta now.

Intensive culture in snakehead connected to increasing in culture stocking density. This result in ordinarily out-breaks of diseases in aquatic animals which is quite disadvantageous for aquaculture activity. Some disease agents affecting to snakehead was recorded, including of bacteria, parasites and fungi that influenced to farmer's income.

Objectives of this study are to define disease agents infecting cultured striped snakehead (*Channa striata*), and to study water quality in snakehead culture ponds. These results will provide the basic information of disease and water quality problems of snakehead culture.

To achieve these objectives, the following studies were conducted: (1) assessment of water quality in snakehead culture ponds in An Giang, Dong Thap and Can Tho provinces; (2) study on status of snakehead farming in Dong Thap province; (3) isolation and identification of pathogens, which are parasites, fungi and bacteria infecting fingerling and grown-out snakehead in An Giang and Dong Thap provinces; and (4) pathogenicity of fungi and bacteria isolated from fingerling snakehead.

RESULTS

Study 1: Assesment of water quality in snakehead culture ponds

1.1 Introdution

Water quality plays an important role in aquaculture activities because of all activities of aquatic animals were under the water. The degree of intensive culture in aquaculture well developed with higher culture stocking density, more feeding and chemicals utilization. These affect water quality, namely some water quality parameters in culture ponds. Rich-nutrition factor in the water impacts the balance in the relationship between the host, pathogens and environment in a cultured pond. This stimulates for higher

sensitivity in host to the pathogens then diseases outbreaks. Moreover, the cultured snakehead in Mekong Delta were fed with small-sized fish, which affects indirectlty to the fish health. Besides, using more chemicals to prevent and treat diseased fish was and are popular that results in reducing the water quality. However, study on snakehead culture pond's conditions was not considered. Therefore, present study was carried out to asses the water quality in cultured snakehead ponds in An Giang and Dong Thap provinces and to provide the basic information on changing of water quality parameters during culture period, especially in the dry and wet seasons.

1.2 Methodology

The study was conducted in snakehead culture ponds in Long Xuyen and Chau Thanh district (An Giang province) and Lap Vo and Tam Nong district (Dong thap province) and Co Do district (Can Tho city) from February to August, 2010. Some water quality factors, comprising of pH, DO, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ was collected once for month at 8:00-9:00 am in 6 months (Table 1, Table 2 and Table 3). Temperature was measure by thermometer in sampling point. The other factors were sampled by preresentative samples, which were collected at 5 sampling points, mixed and sampled. Samples were frozen by Horiba D-58 machine (manufactured by Japan).

1.3 Results

The results of water quality factors surveyed in An Giang, Dong Thap and Can Tho was illustrated in Table 1, Table 2 and Table 3. The average temperature of snakehead cultured ponds fluctuated from 28.3 to 32.7^{o}C, from 26.7 to 32.5^{o}C and from 28.05 to 28.8^{o}C in An Giang, Dong Thap and Can Tho, respectively. Two main reasons for the small variation of temperature in cultured ponds were the water deep (2.5-4.0 m) and the water exchange time of these ponds (2-4 times/day). Therefore, oscillate amplitude of pond water was less than water temperature in the inlet drainage.

The pH value of snakehead cultured ponds varied from 7.25 to 8.75 which was similar to the value reported by Truong Quoc Phu (2006) that the suitable pH value for fish culture is 6.5–9. The average pH value of cultured ponds in An Giang province flutuated from 7.25-7.81, Dong Thap province from 7.33-7.80 and Can Tho city from 8.18-8.75. The pH value highly fluctuated in the third sampling month of snakehead culture, which constituted 7.25 in An Giang and 7.32 in Dong Thap and the fourth sampling month which was 7.95 in An Giang and 7.63 in Dong Thap. This was explained that fish in the ponds in this period were infected by parasites and bacteria, and pond water were exhanged more time to decrease the organic matter, which was from intensive feeding, and reduce pathogent infections; this resulted in low density of phytoplankton in the ponds. Farmers limed the pond's bank making the pH value in the pond higher than in the inlet drainage with 6.93-7.49 in An Giang and 7.06-7.11 and Dong Thap provinces.

$N\text{-}NH_4^+$ concentration in snakehead culture ponds fluctuated from 1.25 to 1.95 ppm. This value was in suitable value for fish growth (0.2-2 ppm) (Boyd, 1990). In sampling period, the first sampling was lowest value of $N\text{-}NH_4^+$ and gradually increased in continuous months. However, the fluctuation of $N\text{-}NH_4^+$ content was higher than the end of culture period because of more water exchange time. The unequal content of $N\text{-}NH_4^+$ in culture ponds and inlet drainage was recorded, 1.25-1.84 ppm compared to 0.04-0.41 ppm in An Giang province, 1.28-1.95 ppm compared to 0.03-0.08 ppm in Dong Thap province and 1.54-1.59 ppm compared to 0.09-1.62 ppm in Can Tho city.

$N\text{-}NO_2^-$ concentration in culture ponds in An Giang province was 0.066-0.329 ppm, Dong Thap province 0.040-0.209 ppm and Can Tho city 0.03-0.08 ppm. Boyd (1990) recommended the concentration of $N\text{-}NO_2^-$ for fish culture should be lower than 1 ppm. This is concluded that $N\text{-}NO_2^-$ concentration in snakehead culture ponds is optimal. Similarily, $N\text{-}NH_4^-$ concentration was higher in snakehead ponds compared to inlet drainage in An Giang and Dong Thap provinces, while in Can Tho $N\text{-}NH_4^-$ concentration was higher in inlet drainage than in the snakehead ponds.

$N\text{-}NO_3^-$ concentration in sampling ponds was gradually reduced in the end of culture period. $N\text{-}NO_3^-$ concentration in inlet drainage in An Giang (0.033-0.163 ppm), Dong Thap (0.072-0.224 ppm) and Can Tho (0.06-0.07 ppm) was recorded and considerably lower than the concentration in culure ponds in An Giang (0.434–1.735 ppm), Dong Thap (0.266–1.229 ppm) and Can Tho (0.04-0.53 ppm).

In conclusion, the temperature variation in snakehead culture ponds in An Giang and Dong Thap provinces was low. $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$ concentration gradually increased in the end of culture period, while $N\text{-}NO_3^-$ concentration gradually decreased. The sudden fluctuation of water quality, espectively in the second, third and fourth culture months affected snakehead culture health. However, in Can tho city, all water quality parameters increased for the whole culture period.

Study 2: Study on diseases in striped snakehead *(Channa striata)*

2.1 Introduction

Disease problems in aquaculture are essensially considered, espectively in intensive more popular culture systems. The development of culture area and diversification of cultured species that incurred the concerns on seed quality, feed supply, custody skills and management in fish health. In culture condition, diseases ordinarily occur in conditions of high denisity and rich nutrition, which both affect the balance between fish and pathogens and fish will be more sensitive to pathogens (Bui Quang Te, 2006). Snakehead with *Channa striata* and *Channa micropelte* were cultured popularly in Mekong Delta with many kinds of systems such as pond culture, hapas culture and cage culture. In recent years, studies on snakehead was carried out by Le Xuan Sinh and Do Minh Chung, 2009; Nguyen Thi Diep Thuy, 2010; and Sarowar *et al.*, 2010. These studies were provided the data on general information of snakehead culture systems as well as information of disease problems that occurred in cultured snakehead. Basing on the previous studies, some pathogens were confirmed infecting snakehead, comprising of parasites, fungi and bacteria. The prevalence infection of each pathogen was different and based on the culture conditions. However, a specific study on the pathogens that infecting to snakehead in two provinces of An Giang and Dong Thap was not carried out. Therefore, this study was conducted to determine the sorts of pathogen infecting to snakehead in rearing and culturing conditions.

2.1 Methodology

2.1.1 Investigation on status of snakehead farming in Dong Thap province

The investigation was carried out from February to May, 2011 in Tam Nong district, Dong Thap province.

Methods of secondary data collecting: secondary data were collected from the reports of local agencies and sectors consisted of Fisheries Stations of Tam Nong (Dong Thap provine), Department of Fisheries Resources Management of Dong Thap province, newspapers and magazines of aquaculture, websites of aquaculture and relating aquaculture documentations.

Methods of primarily data collecting: primarily data were recorded by directly interviews with 20 farmers in Dong Thap province using questionnare with information on farmer's information, culture skills, custody and fish health management and status of diseases out breaks.

2.1.2 Study on parasites infecting reared and cultured snakehead fish

The study on parasites infecting reared and cultured snakehead fish was performed from 02-05/2011 and 02-08/2010, respectively, in An Giang and Dong Thap. Parasites were identified basing on morphology that was described by Ha Ky and Bui Quang Te (2007). Moreover, the prevalence of parasite infection and infection intensity of parasites were calculated basing on Dogiel (1960) (cited by Nguyen Thi Thu Hang *et al.*, 2008)

Methods of identification of ectoparasites: wet smear of muscus in skin and fin and examined under microscope with X 200 magnification. When parasites were observed the camera was used to take pictures and to identify.

Methods of identification of indoparasites: fish were operated and segrerated internal organs (intestine and stomach) successively. Wet smear was used and observed under microscope. Parasites presented in the smear, photographs were taken and parasites were identified and classified.

2.1.3 Study on fungi infecting reared and cultured snakehead fish

Study samples were collected from 03-04/2011 (reared fish) and from 02-08/2010 (cultured fish) in An Giang and Dong Thap provinces. Samples were isolated and identified in the Department of Aquatic Biology and Pathology, College of Aquaculture and Fisheries, Can Tho University.

Wet mount observation: fish apparent was observed by naked eyes with cotton-like in fish skin; wet mount observation was carried out based on Gam *et al*. (1980) and Hatai *et al*. (2000). Wet mount preparation was described by cutting a small specimen with a drop of cotton-blue stain in the slide and observing under a light microscope in magnification of x200 or x400 to define the existence of hyphae or conidia.

Isolation: wet mount observation with existence of fungi, fish samples was performed to isolate fungi. The procedure was illustrated by steps: 2 mm diameter of specimen was washed 3 times in sterile physiological saline; and sample was inoculated on GYA (1% glucose, 0.25% yeast-extract and 1.5% agar). Ampicilin and streptomycin were added with 500μg/ml for each to the medium to inhibit bacterial growth. Plates were incubated at 25-30°C for 1-4 days and subcultured into fresh GYA plates in 3 times to have pured fungal isolates (Hatai *et al*., 2000).

Identification: fungi were identified based on Coker (1923), Johnson (1956), Scott (1961) and Seymour (1970) (for lower fungi) and de Hoog *et al.* (2000) (for higher fungi) with morphological characteristics.

2.1.4 Study on bacteria infecting reared and cultured snakehead fish

Fish samples were collected in culture ponds in An Giang and Dong Thap provinces from February to August, 2010. Samples were isolated and identified at the Department of Aquatic Biology and Pathology, College of Aquaculture and Fisheries, Can Tho University.

Isolation: fish were killed and weighted. Observation by naked eyes and disease syndromes were recorded. Fish were cleaned by alcohol (70%) and then samples were taken by incision in the infectinous area or internal organs (liver, kidney, spleen) with sterile incision knife. Samples were taken by sterile loop and culture on the TSA medium plates. Plates were incubated at 28°C for 24-48 hours. Recoding coloni colour, shape and subculture were performed until pure culture plates.

Identification: bacterial strains were identified acording to methods of Barrow and Feltham (1993) and test kit API 20E, manufactured by BIO MÉRIEUX company, was used to identify based on morphological and biochemical characteristics.

2.1.5 Pathogenicity

a. Pathogenicity to snakehead of *Achlya* sp. VN1101

Experimental fish: snakehead (10 g/fish) were collected in reared pond in Can Tho. 50 fish were acclimatized in fibreglass tanks for one week before experiment started at the College of Aquaculture and Fisheries, Can Tho University in April, 2011. Fish were fed with commercial feed to satisfaction.

Fungal preparation: isolate of *Achlya* sp. VN1101 was isolated in diseased snakehead fingerling in clinical sign of cotton-like in the fish body. Fungal specimen of *Achlya* sp. VN1101 was cultured in GY broth (1% glucose, 0,25% yeast-extract) at 28^{o}C in 2-3 days, rinsed 3 times in sterile tap water and placed in 25 ml of sterile tap water in 18 hours. The spore suspensions were filtered throught two layers of sterile medical gauze to obtain spore suspension without hyphae. The number of spore was defined by counting in a haemocytometer and adjusted to $5x10^5$ (high dose) and $2.5x10^3$ (low dose) spores/ml.

Pathogenicity test: the experiment included 3 treatments which were 1 control treatment (1 replicate) and 2 experimental treatment (2 replicates). Plastic tanks was used with 10 individuals and 20 L of fresh water for each. Fish were directly inoculated into the left dorsal trunk muscle under dorsal fin of 0.1 ml of spore suspension in experiment group and 0.1 ml sterile distilled water in control group. The challenged fish were fed by commercial feed daily two times. Challenged fish were observed twice a day and noted the disease symptom and mortality. Re-isolation and re-identification were carried out in diseased fish observed.

b. Pathogenicity to snakehead of *Aeromonas hydrophila*

Experimental fish: snakehead (20±5.0 g/fish) were collected in reared pond in Can Tho. 180 fish were acclimatized in fibreglass tanks (250 L) one week before experiment started at the College of Aquaculture and Fisheries, Can Tho University in May, 2011. Fish were fed with commercial feed to satiation. Bacterial preparation: Aeromonas hydrophila CĐ1012 was isolated from diseased snakehead fish with separation and hemorrhage in the fish body in Co Do district, Can Tho city. Bacterial strain was cultured in agar plates at 28^{o}C in 24h, centrifuged in 3 times and suspended in sterile physiological saline (0.85% NaCl). Ten-fold dilution method was used to adjust the bacterial suspension with desired dilution. The concentrations of bacteria were $3.67x10^4$, $3.67x10^5$, $3.67x10^6$, $3.67x10^7$ CFU/ml which was used to form the challenge test.

Pathogenicity test: the challenge test comprised 6 treatments corresponding to 1 negative control test, 1 possitive control test and 4 concentrations of bacterial suspension. 15 fish were introduced into each tank. Each treatment composed 2 replicates and was randomly set. Each fish was injected with 0.1 ml of bacterial suspension in the peritoneum in experiement group and 0.1 ml sterile sanility in positive control treatment and without injection to negative control treatment. Fish were not fed during the experiment period of 14 days. Fish activity and mortality were noted. Lethargic fish were sampled, re-isolated and re-identified by traditional method and PCR technique.

2.2 Results and discussions

2.2.1 Investigation on status of snakehead farming in Dong Thap province

Culture system: snakehead culture systems in Dong Thap province were well developed (Fig. 1). Hapa system with different sizes placed in eathern ponds was effective system because of being easy in manage of water quality and feeding. Almost ponds had feed tray for holding feed for snakehead to catch. Snakehead feed was commonly small-sized fish.

Age and education level of snakehead farm owners: the average age of owners was 44.7±9,12 years old. The minimum and maximum age of the owners was 26 and 60 years old, respectively. The age of 40-60 years old contributed to 70% and under 40 year old was remaining. Education level of owners was primary (60%), secondary (20%) and senior high school (20%).

Experiences and culture skill improvement: the results of the investigation showed the average number of years farmers spent for culturing snakehead was 9±4,4, and the farmers having shortest experience was 4 years and longest experience was 20 years. Although, difficulties in culture commonly happened by the water pollution and diseases outbreaks. The owners improved culture skills based on the former's

experiences and attending additional trainings provided by provincial fisheries officers, which constituted 50% of total farmers.

Water quality management: 75% of owners used test kit for testing key water quality parameters and 25% was sensory. Water exchange in culture ponds was carried out directly from rivers without any treatment. This maybe cause difficulty in control of fish disease outbreaks.

Status of diseases in snakehead fingerling: some common disease outbreaks in snakehead fingerling were illustrated in Fig. 2. Parasites infecting the fish was recorded in a very high proportion of 70%. Parasite infection in culture fish usually occured in the period of April to October. Moreover, fungal infection leading to 30-50% fish mortlity occurred from August to November annually. Futhermore, bacterial infection regconized from April to August, which resulted in 15-40% mortality. White spot in fish liver has just recorded, and was harmful to culture fish, causing high mortality. Treatment of such disease was difficult and farmers lost their production and income. The common methods used for treatment of diseased fish was water exchange and chemicals utilization such as sulfate copper ($CuSO_4$) used for parasite infection disease and iodine for fungal infection disease.

2.2.2 Study on parasites infecting reared and cultured snakehead fish
a. snakehead fingerlings in nursery ponds
Total of 142 samples were identified for parasite infection on snakehead fingerlings. The results showed that 8 parasite genera, of which 5 ectoparasite genera and 3 indoparasite genera, were identified and classified from snakehead fingerlings.

Ectoparasites
Ectoparasites infecting snakehead fingerlings included *Trichodina*, *Epistylis*, *Chilodonella*, *Dactylogyrus*, *Trianchoratus* (Fig. 2). The main infected organs were fish skin and gills.

Trichodina: microscopic observation was demonstrated *Trichodina* was classified with saucer-shaped, adhesive disc concave with denticulate ring (Fig. 2A), similar to the description of Shwani *et al.* (2010). The prevalence infection and infection intensity of *Trichodina* were 93.7% and ++, respectively. *Trichodina* probably damage snakehead fingerling. These findings agree with Ha Ky and Bui Quang Te (2007) results of the prevalence infection and infection intensity of *Trichodina*, with 90-100% and 50-100 individuals/len.

Epistylis: morphology of *Epistilis* was observed with bell-shaped, oral disc and collar and a stalk; Epistilis branch from parasitic area (Fig. 2B). The prevelance of infection with *Epistylis* was 71.8% and infection intensity was ++; in this situation, *Epistilis* migh affect snakehead fingerlings. However, *Epistilis* ordinarily estimated as an indicator organism to assess water quality (Nguyen Thi Thu Hang *et al.,* 2008). Therefore, the presence of *Epistilis* in culture conditions should be linked to poor water quality of snakehead ponds.

Henneguya: Henneguya was observed in the wet smear which was described as ellipsoidal shape, a polar capsule and a tail (Fig. 2C). Henneguya was also recorded by Azevedo and Matos (1996). The prevelance of infection was 3.52%, being lower than the rate rported by Azevedo and Matos (1996) in *Hoplias malabaricus* (6.7%) and the infection intensity was low (+), probably not affecting snakehead fingerlings.

Chilodonella: Chilodonella is a oval to foliate shape. The body has ciliary band in the ventral right side (Fig. 2D). Morphological characteristics of *Chilodonella* was illustrated by Reda (2011). The prevelance of *Chilodonella* in snakehead fingerling was 13.4% and intensity of infection was low (+). Therefore *Chilodonella* did not affect snakehead fingerlings and cause fish mortality and was not harmful to fish health.

Trianchoratus: the body is tube-shaped, eyespots and adhesive disc which included of 7 pairs of margin hooks and 2 pairs of central hooks (Fig. 2E). *Trianchoratus* was noted as a parasite in snakehead in Thailand with infection prevelannce was 40% and intensity was 13 (Theerawoot, 2008), showing a higher infection rate than this study (5.63%) and lower infection intensity (+).

Gyrodactylus: there is a similarity in morphological characteristics between *Trianchoratus* and *Gyrodactylus*, but no eyespot is found in *Gyrodactylus.* Adhesive disc has 2 ventral hooks and 16 marginal hooks (Fig. 2F). The prevalence of infection was 12.7% and intensity was 4 individuals/slide. Based on the prevalence and intensity, *Gyrodactylus* was probably non-affective to snakehead fingerlings.

Indoparasites

Indoparasites were identified in snakehead fingerlings, comprising of 5 genera with *Proteocephalus*, *Spinitectus*, *Pallisentis* (Fig. 3). Indoparasites parasite on fish intestine and stomach.

Spinitectus: Spinitectus colonize in fish stomach. The body is cylindrical, bifurcate distal tip of spicule, of which 16-26 spines for each and the number of spicule increasingly in body endian. *Spinitectus* body was observed with tapering posterior end (Fig. 3C). The prevalence of *Spinitectus* was 19% and intensity was 15 individuals/stomach. Higher infection prevalence (63.8%) and lower intensity (1 individual/ stomach) was report in knife-fish by Bui Quang Te (2001).

Pallisentis: Pallisentis parasite in fish intestine. *Pallisentis* body is cylindrical, vermiform, with a knob-like anterior and a tapering posterior end. The anterior portion includes proboscis, comprising of 4 circles of hook with 10 hooks in each circle. This was also described by Farooqi (1958) in freshwater eel. The prevalence of *Pallisentis* detected from snakehead fingerlings was 16.9% and intensity was 2-11 individuals/intestine.

Proteocephalus: Proteocephalus was found in fish intestine. The body is cylindrical, aspinose with 4 suckers and lot of smaller hooks surrounding apical disc (Fig. 3B). *Proteocephalus* was also regnonized in *Sinocyclocheilus grahami* by Pin (1997). The prevalence and intensity of *Proteocephalus* detected from snakehead fingerlings was 3,52% and 5 individuals/slide, respectively.

b. Cultured snakehead in grow-out ponds

Total of 296 samples of grown-out snakehead were collected. The results showed that 23 parasite genera were detected (Table 4). Infested area was different in different parasites which were demonstrated in Table 5. The present study was noted the prevalence infection and intensity infection of parasites in grown-out snakehead were considerably high. *Gyrodactylus* infested with highest prevalence infection of 72.6% and intensity of 13 indiciduals/slide on fish skin. However, *Lamproglena* infested with lowest prevalence and intensity of 0.7% and 2 individuals/slide, respectively. Parasite infection was noted in correlating to bacterial infection in ordinarily. In conclusion, parasites was a secondary pathogen that infested to grown-out snakehead.

Protozoa infested to grown-out snakehead included of *Myxobolus, Trichodina, Chilodonella, Epistylis, Tripartiella, Apiosoma, Henneguya*. Genus of protozoa colonized on fish skin and gills. Infection was usually recorded in the early months of cultured period. These parasites were also reported by Bui Quang Te (2001),

Myxobolus and Henneguya: Myxobolus infested fish with prevalence of infection was 37.2% and intensity was low (+). Sitja-Bobadilla *et al.* (1993) assesed that *Myxobolus* parasites on organs of freshwater fish with prevalence of 68%. Moreover, prevalence of *Myxobolus* in carp which was 96% was regconized (Ha Ky and Bui Quang Te, 2000).

Henneguya was found in snakehead gills in the first and third sampling months of culture period (in An Giang province) and the second month (in Dong Thap province). The prevalence of infection was 4.4% and intensity was low (+). This genus, however, was not found other samples. In Mekong Delta, *Henneguya* infested *Anabas testudineus* and *Ophiocephalus micropeltes* with average prevalence of infection of 66.7% and high intensity (Ha Ky and Bui Quang Te, 2007). So, the infection prevalence and intensity of this genus in this study did not affect cultured snakehead in An Giang and Dong Thap provinces.

Trichodina and Tripartiella: Trichodina and *Tripartiella* belong to the Family *Trichodonidae*, Order *Mobilina* (Ha Ky and Bui Quang Te, 2007). The prevalence of infection and intensity of *Trichodina* and *Tripartiella* in grown-out snakehead was 31.8% and 14.2%, respectively. The intensity of both parasites was low (+). *Trichodina* and *Tripartiella* were found in gills and skin which contributed low percentage. The infection of *Trichodina* and *Tripartiella* in this study did not affect grown-out snakehead because of the lower prevalence and intensity infection. However, Ha Ky and Bui Quang Te (2000) reported that fish were infected with 90-100% and 50-100 individuals/slide of prevalence and intensity, respectively.

Chilodonella: Chilodonella belong to the Family *Chilodonellidae,* Order *Cyrtophorida* (Ha Ky and Bui Quang Te, 2007). *Chilodonella* was found in fish gills in the second and third sampling month in An Giang and Dong Thap provinces, respectively. The prevalence of infection was 1.7% and the intensity was high with +++. Arthur and Te (2006) reported that *Chilodonella* exists in culture condition when the water quality is poor and stocking density was high. This estimation was similar to snakehead culture condition in sampling time. However, it is concluded that *Chilodonella* was not an important pathogen infecting grown-out snakehead.

Epistylis and *Apiosoma: Epistylis* and *Apiosoma* belong the Family *Epistylididae*, Order *Peritrichida* (Ha Ky and Bui Quang Te, 2007). *Epistylis* and *Apiosoma* was found from the second to fouth sampling month in An Giang province and in the first sampling month in Dong Thap province. The prevalence and intensity of *Epistilis* and *Apiosoma* in cultured snakehead was 17.2% and ++ and 13.9% and +, respectively. The prevalence and intensity of *Epistilis* and *Apiosoma* of this study was lower than the study of Nguyen Thi Thu Hang *et al.* (2008) in catfish: 2.7% and 30 individuals/len and 5.3% and 7 individuals/len, respectively.

Trianchoratus: Trianchoratus belong to the Family *Ancyrocephalidae,* Order *Dactylogyridea* (Ha Ky and Bui Quang Te, 2007). *Trianchoratus* was found in snakehead gills. Bui Quang Te and Vu Thi Tam (2000) concluded that *Trianchoratus* parasite on fish gills was advantageous for other pathogens such as fungi, bacteria and other organisms.

Gyrodactylus: Gyrodactylus classificated to the Family *Gyrodactylidae* (Ha Ky and Bui Quang Te, 2007). The prevalence and intensity of infection of *Gyrodactylus* were 72.6% and 6 individuals/slide, respectively. *Gyrodactylus* was found in fish skin and gills.

Cestoidea: Cestoidea were found in fish intestine in the third sampling month (in An Giang province) and the second sampling month (in Dong Thap provin). The prevalence and intensity of infection of *Cestoidea* were 1.4% and 1 individuals/intestine. Similar findings were reported by Ha Ky and Bui Quang Te (2007) in *Polyonchobothrium* (Fig. 3A) colony in fish intestine and liver in Mekong Delta with low prevalence (7.4%) and intensity (1-3 individuals/intestine). In this study, the prevalence and intensity of infection of *Proteocephalus* were 2.0% and 2 individuals/intestine, respectively. *Proteocephalus* mainly presented in fish intestine which sampled in the second sampling month (An Giang) and the third sampling month (Dong Thap).

Trematoda: Exochis belong to the Family *Cryptogonimidae*, Order *Opisthorchiida* (Ha Ky and Bui Quang Te, 2007). Metacercaria of *Exochis* was detected only one time in the fourth sampling month (in An Giang) in fish fins, with prevalence of 3.0% and intensity of 2 individuals/slide. In addition, Metacercaria of *Clonorchis* was also found in the fish fins, with prevalence of 3.7% and intensity of 2 individuals/slide. *Clonorchis* (Fig. 10B) belong to the Family *Opisthorchidae*, Order *Opisthorchiida* (Ha Ky and Bui Quang Te, 2007). Moreover, the spore of *Haplorchis* was found in fish fins in the second sampling month (An Giang), with prevalence of 5.1% and intensity of 3 individuals/slide. *Haplorchis* belong to the Family *Galactosomidae*, Order *Strigeata* (Ha Ky and Bui Quang Te, 2007). Thien *et al.* (2009) revealed that this parasite infest fish in the stage of Metacercaria. This agrees with the present study. Scolex infection to grown-out snakehead was in stage of Metacercaria, with small spot in fish muscle. Furthermore, scolex also infected snakehead cultured in Nam Binh and Nam Đinh provinces with higher prevalenc (18.5%) and intensity (21-50 individuals/slide) (Kino *et al.*, 1998; Ha Ky and Bui Quang Te, 2007).

Nematoda: Nematoda was found in fish stomach, only *Neocamallanus* in both fish stomach and intestine. *Spinitectus* infested snakehead with prevalence of 17.9% and intensity of 5 individuals/stomach. *Spinitectus* was found in all sampling periods, with healthy and unhealthy fish. *Neocamallanus* existed in snakehead intestine, stomach and bladder (Fig. 3D). The prevalence and intensity of *Neocamallanus* was 4.7% and 1-3 individuals/organ, respectively. Moreover, *Gnathostoma* and *Capillaria* was found only in the second and fourth sampling month in An Giang province with prevalence of 1.4% and 1.0% and intensity of 2 individuals/stomach and 1 individual/stomach, respectively (Fig. 3E and 3F). *Gnathostoma* was found in snakehead stomach for the first time. In addition, *Capillaria* parasites were mainly found on fish fingerling (Moravec *et al.*, 2001). This agrees with our findings.

Acanthocephala: Pallisentis was found in fish intestine in sampling months of 1, 2, 3, 5 (An Giang) and 1, 2, 4, 6 (Dong Thap). The prevalence of infection of *Pallisentis* was 27.4% and 2 individuals/intestine, respectively. *Pallisentis* belong to the Family *Quadrigyridae*, Order *Acanthogyrida* (Ha Ky and Bui Quang Te, 2007). Bui Quang Te (2001), reported that *Pallisentis* colonized in intestine of snakehead with the prevalence of 20% and intensity of 3-53 individuals/intestine.

Crustacea: Ergasilus existed in snakehead gills with the prevalence of 6.4% and intensity of 2 individuals/fish. *Ergasilus* belong to the Family *Ergasilidae,* Order *Copepoda* (Ha Ky and Bui Quang Te, 2007). *Ergasilus* was found in the second sampling month in both An Giang and Dong Thap provinces. *Ergasilus* was noted by Yunia *et al.*, 2007 that they infected freshwater and marine fish. Ha Ky and Bui Quang Te (2007) regconized *Ergasilus* infecting snakehead gills, with the prevalence of 11.3% and intensity of 2 individuals/fish. This result showed a lower prevalence and intensity infection of *Ergasilus* in cultured snakehead in An Giang and Dong Thap provinces.
Besides, *Lamproglena* was found in fish gills, with with the prevalence of 0.7% and intensity of 2 individuals/fish. *Lamproglena* was only found in the second sampling month in An Giang province. *Lamproglena chinensis* not only infected snakehead but also knife-fish and climbing perch with higher prevalence of 5,6% and similar intensity (Tran Ngoc Tuan, 2010).
Moreover, *Lernaea* was found in the sampling months of 1 and 4 (An Giang) and 3 (Dong Thap). The prevalence of infection of *Lernaea* was 2.0% and 1 individual/fish, respectively. Kabata (1981) defined that *Lernaea* infected different stages of freshwater fish, but mainly in fingerling stage. In addition, *Argulus* was observed in the sampling months of 3 and 4 in An Giang and Dong Thap provinces, with the prevalence of 4.7% and intensity of 2 individuals/fish. In the previous study, *Argulus chinensis* was recorded in *Channa striata* and *Ophiocephalus micropeltes (*0.4-0.8 kg), with the intensity of 30-50 individuals/fish, which cause high fish mortality (Bui Quang Te, 2008). Therefore, *Argulus* may be harmful to grown-out snakehead.

2.2.3 Study on fungi infecting snakehead fingerlings and grown-out snakehead

a. Snakehead fingerlings in nursey ponds

Isolation and identification

Total of 14 isolates of fungi were isolated from snakehead fingerlings, with clinical signs (Fig. 4). Fungal isolates were named from VN1101 to VN1114 (Table 6). The morphological characteristics of fungal isolates were similarity. The isolates VN1101 was studied for morphology. The colony grew rapidly on GYA medium at 28^{o}C with 60 mm in diameter (in 3-4 days of incubation). Colony colour was whitish. Aerial mycelium was abundant and dense (Fig. 5A). The hyphae was stout, non-septate and apical zoosporangia formation. Zoospores were spherical shape produced in zooaporangium (Fig. 5B). Zoospores release and aggregate at the tip of zoosporangium as irregular ball (Fig. 5D). Cysts rise to secondary zoospores, further encyst and then germinate into hyphae (Fig. 5E). Based on morphological characteristics, comprising of hyphae and zoospores and the key of Coker (1923) identificated that the isolates VN1101 isolated from snakehead fingerling in Dong Thap province belonged to genera of *Achlya*. Frequency of genera of *Achlya* was 46%. *Achlya* belong to the Class *Oomycetes*, Order *Saprolegniales*, Family *Saprolegniaceae* (Neish and Hughes, 1980).

Pathogenicity of isolate *Achlya* sp. VN1101 isolated from snakehead fingerling with clinical signs of fungal infection was carried out (Fig. 6). After 7 days of challenged test, the result of experiment was recorded, clinical signs (Fig. 7A) and mortality were considerably high in both low dose and high dose, which were illustrated in Table 8. Moreover, re-isolation and re-identification of fungi in challenged fish were performed. The results showed that *Achlya* was isolated from unhealthy fish (Fig. 7B). However, no fungi was isolated from healthy fish in control treatment. Therefore, *Achlya* sp. VN1101 was primitive pathogen in cotton-like disease in snakehead fingerlings (5-10 g/fish).

b. Cultured snakehead in grow-out ponds

Isolation and identification

14 isolates fungi, including of 3 isolates of lower fungi and 11 isolates of higher fungi, were isolated from grown-out snakehead with clinical signs (Table 6). Basing on morphological characteristics, growth ability and hypae and conidia characteristics, fungal isolates identified were genera *Achlya* (A001, A002 và A003), genera *Fusarium* (Fu001 và Fu002), genera *Acremonium* (Ac001, Ac002, Ac003, Ac004 và Ac005) and genera *Geotrichum* (Ge001, Ge002, Ge003 và Ge004), which were showed in Table 5. However, no fungi were isolated from snakehead without clinical signs. The Figure 16 showed the frequency of fungal isolates isolated from grown-out snakehead.

Achlya

The clinical signs of cultured snakehead infested by *Achlya* showed reduction in appetite, abnormal body, floating on the water surface or the head out of the water, darker skin and ulcerative formation. The mycelium grow in the infectinous places with whitish colour and cotton-like. Snakehead samples, weighting of 96.6±38.4 g to 165±28.6 g, were collected in culture ponds in An Giang and Dong Thap provinces. *Achlya* was isolated only in the first sampling month in ponds.

Fungal colony grew quickly on GYA at 28^{o}C with 58 mm in diameter after 4 days incubated. Colony had white colour with aerial mycelium, which were white and floccose. The hyphae were stout and aseptate. Cultivation on low nutrient medium, after 18 hours, the hyphae grew rapidly with a septate nearly to the hyphal tip and formed spores agglomerating in portion of septate to hyphal tip. Sporangium was 11.5±0.68x3.8±2.83 µm in size. Zoospores released and clustered in the sporangium tip. Spores were spherical or elipsoid with flagella and good swimmers. Based on morphological characteristics and the classification key of Scott (1961), fungi isolates isolated from cultured snakehead was genera *Achlya* with frequency of 21.4%. *Achlya* belong to *Oomycetes*, the Family *Saprolegniaceae*, Order *Saprolegniales* (Scott, 1961).

Acremonium

Snakehead were infected by *Acremonium* in cultured ponds, with body weight of 244±42.3 g to 313±18.3 g. Clinical signs were recorded with swimming along pond banks, dense muscus on the body surface and white spot in whole body (Fig. 8A). *Acremonium* was isolated from fish skin in the second sampling month in An Giang and the second and third month in Dong Thap.

Colony grew rapidly on GYA at 28^{o}C with 40 mm in diameter after 6 days of incubation. Fungal colony was applenate and smooth. Colony colour was white in margin and yelowish in center (Fig. 8B). The hyphae were septate, hyaline, branching and dense with 1-2 µm (Fig. 8C). Conidia were normally 1-cell, hyaline, spherical or ellipsoid shape with 3.1±0.82x1.3±0.22 µm, accumulate in conodiophore apex (Fig. 8D). Conidiospore, branched from creeping hyphae, was phialide shape, tapering toward ot the apex with visible collarete and casually septate visibility in the basal cells (Fig. 8E). Based on morphological chacteristics and classification key of de Hoog *et al.* (2000), fungal isolated from cultured snakehead was belonging to genera *Acremonium* with frequency of 35.7%. *Acremonium* belong to Class *Hyphomycete,* Family *Hypocreaceae*, Order *Hypocreales* (de Hoog *et al.*, 2000).

Acremonium was recorded as a pathogent in black gill disease in marine crustacean (Duc *et al.*, 2009). In previous study, *Acremonium* sp. was isolated from climbing perch with fungal infection" (Tran Ngoc Tuan, 2010). This is the first time *Acremonium* was isolated from cultured snakehead in An Giang and Dong Thap province.

Fusarium

Snakehead were infected by *Fusarium* in cultured ponds in weighting of 258±32.3 g to 288±17.5 g. Clinical signs were recorded with poor swim, swim with head out of water, dense muscus on the body surface, scabrous scales, swelling adomen and haemorrhage in visceral organs (Fig. 9A). *Fusarium* was isolated from cultured snakehead in the second and the third sampling month in An Giang and the second and third month in Dong Thap.

Colony grew rapidly on GYA at 28^{o}C with 46 mm in diameter after 6 days of incubation. Fungal colony was applenate and smooth. Colony colour was white, pinkish (Fig. 9B). The aerial mycelium were white, abundant and softly floccose. The hyphae were septate and branching with 2.3-5.2 µm in diameter (Fig. 9C). Macroconidia were 1-5 septate, dosiventral, falcate, slightly curved with sometimes foot-shape basal cells, 19.8±3.61x2.9±0.63 µm in size (Fig. 9E). Basing on morphological chacteristics and key of de Hoog *et al.* (2000), fungal isolated from cultured snakehead was belonging to genera *Fusarium* with frequency of 14.3%. *Fusarium* belong to the Class *Hyphomycete,* Family *Hypocreaceae*, Order *Hypocreales* (de Hoog *et al.*, 2000).

In similar to *Acremonium*, *Fusarium* was studied in black gills of marine crustacean (Alderman and Polglase, 1985; Rhoobunjongde *et al.*, 1991; Khoa *et al.*, 2004; Khoa and Hatai, 2005). Moreover, according to Theo nghiên cứu của Hatai *et al.* (1986), *Fusarium* was also a main pathogen infecting red sea bream *Sparagus* sp. with white spots in the body surface (Hatai *et al.*, 1986). In addition, *Fusarium* was isolated from climing perch *Anabas testudineus* with fungal infection (Tran Ngoc Tuan, 2010). This is the first time *Fusarium* was isolated in cultured snakehead in An Giang and Dong Thap provinces.

Geotrichum

Geotrichum was recorded infecting snakehead of 258±18.6 g to 338±23.3 g in the second and third sampling month in An Giang and Dong thap provinces. Fish were noted to the clinical signs with poor swimmers, exothalmic, dense muscus in body surface, white spot in body and scabrous scales (Fig. 10A and 10B).

Colony grew on GYA at 28^{o}C with 35 mm in diameter after 6 days of incubation. Fungal colony was applenate, smooth and yelowish in colony center (Fig. 10C). The hyphae were hyaline, branching and septate (Fig 10D). Conidia were formed in the hyphae tip, conidiophores, or by arthroconidia (Fig. 10E and 10F). Conidia were hyaline, 0-3 septate, spherical or cylindrical shape with shape with 8.6±2.28x2.6±0.81 µm (Fig. 10F). Basing on morphological chacteristics and key of de Hoog *et al.* (2000), fungal isolated from cultured snakehead was belonging to genera *Geotrichum* with frequency of 28.6%. *Geotrichum* belong to the Class *Hemiascomycetes*, Family *Dipodascaceae,* Order *Saccharomycetaceae* (de Hoog *et al.*, 2000).

In previous sudy, *Geotrichum* sp. was isolated from fungal infection in climbing perch with clinical signs of scabrous scales and dense muscus in the fish body (Tran Ngoc Tuan, 2010). Such clinical signs were also recorded in this study.

2.2.2.4 Study on bacteria infecting reared and cultured snakehead fish

Total of 296 snakehead samples was isolated and identified to 81 strains of bacteria infecting cultured snakehead with the clinical signs. Based on morphological characteristics and biochemical and physiological tests, bacterial strains were classified in 5 genera (Table 7), including of *Aeromonas* (38.3% in frequency of apparence)*, Edwardsiella* (17.3%)*, Vibrio* (16.0%), *Streptococcus* (14.8%) and *Pseudomonas* (13.6%). However, no bacterial strains were isolated from healthy fish. The results showed that *Aeromonas* was isolated during the whole culture period, *Edwardsiella* was ordinarily isolated in the second sampling month and *Streptococcus* was only isolated in market size (the fifth sampling month).

Aeromonas

Total of 31 *Aeromonas* strains were isolated from cultured snakehead in An Giang (whole sampling period or 6 sampling months) and Dong Thap (5 sampling months) provinces. The clinical signs were recorded with poor swimming fish, haemorrhage and ulcerative lesion in the fish body, haemorrhage in fish fins, oral cavity and fish tongue (Fig. 11A). Moreover, red fluid with smell, bruising liver, haemorrhage in stomach, kidney and blader and intestine without feeds were noted in operating of abdominal cavity (Fig. 11B).

Bacterial colony grew rapidly on TSA at 28^{o}C with round, whitish, slightly convex (Fig. 11C). Biochemical tests indicated that Gram-negative, rod-shape (Fig. 11D), motile, oxidase and catalase-positive, O/F-fermenting and O/129-inhibiting. Based on morphological characteristics, biochemical tests and classification key of Barrow and Feltham (1993) defined bacterial strains isolated from cultured snakehead was *Aeromonas* with frequency of apparence of 38.3%. *Aeromonas* belong to the Family *Aeromonadaceae*, Order *Aeromonadales*. The exam of API 20E kit on biochemical properties identified two isolates of 9 and 74 were *Aeromonas hydrophila*, but some differences were demonstrated by API 20E kit on the other strains, which were classified to genus of *Aeromonas.*

Aeromonas hydrophila was recorded as a main pathogen causing of haemorrage in cage-culture of basa catfish, carp and walking catfish with the same clinical signs shown in present study (Tanasomwang and Saitanu, 1979; Angka, 1990). Therefore, *Aeromonas* was a probable pathogen causing haemorrhagic signs in cultured snakehead in An Giang and Dong Thap provinces.

Pathogenicity to fingerling snakehead of *Aeromonas hydrophila* was carried out with some biochemical characteristics, which were illustrated in Table 8. The results of experiment on pathogenicity of *Aeromomas hydrophila* to snakehead showed similarl clinical signs of natural snakehead infection and the cumulative mortality was noted (Fig. 12). Died fish were noted in all concentration of bacterial suspension excepting to negative control treatment. The concentration of 3,67x10^{6} CFU/ml of bacteria showed the earliest time of fish dead was 15 hours after injection. In contrast, the longest was 37 hour in treatmet of concentration of 3.67x10^{4} CFU/ml of bacterial suspension. The present study result was

similar to a previous study result of Rahman *et al.* (2004) in injecting *A. hydrophila* to *Channa punctatus* that the result showed LD_{50} of bacterial suspension was $3{,}42x10^7$ CFU/ml after 15 days. Highest mortality was 100% when injecting to bacterial concentration of $3.42x10^9$ CFU/ml. On the contrary, there were no fish was observed in the bacterial concentration of 10^5 CFU/ml. Therefore, *A. hydrophila* was defined as a main pathogen that caused haemorrhage in snakehead fingerlings by the traditional identification methods (Fig. 13) and PCR technique applied in which bacterial strains re-isolated had a band of 209 bp (Fig. 14).

Edwardsiella

Total of 14 *Edwardsiella* strains were isolated from cultured snakehead in the only the second sampling month in An Giang and Dong Thap provinces. The fish samples weighted 150-280 g/fish. The clinical signs were recorded with poor swimming fish, pale body sign and ulcerative lesion in the fish body with smell (Fig. 15A). Moreover, white spots existing in liver, kidney and spleen were noted in abdominal cavity (Fig. 15B).

Samples were taken and cultured on TSA and incubated at 28^{o}C in 48 hours. Bacterial colony grew quickly with whitish colour (Fig. 15C). Biochemical tests indicated that Gram-negative (Fig. 15D), rod-shape, oxidase-negative, catalase-positive and O/F-fermenting. Based on morphological characteristics, biochemical tests and classification key of Barrow and Feltham (1993) identification of bacterial strains isolated from cultured snakehead was *Edwardsiellas* with frequency of apparence of 17.3%. *Edwardsiella* belong to the Family *Enterobacteriaceae*, Order *Enterobacteriales*. API 20E kit was examined that identification of two isolates of 58 and 80 were *Edwardsiella tarda*, but some differences were demonstrated by API 20E kit on the other strains, which were classified to genus of *Edwardsiella*.

Edwardsiella ictaluri was isolated in the first time on chanel catfish *Ictalurus punctatus* (Hawke, 1979). Furthermore, *E. ictaluri* was noted to cause white spot clinical sign on other catfish *Ictalurus fuscatus*, *Ictalurus catus* and *Pangasianodon hyphothalmus* (Plunb and Sanchez, 1983; Furguson *et al.,* 2001). This species were also detected in cultured snakehead fish in the Mekong delta.

Vibrio

Total of 13 *Vibrio* strains were isolated from cultured snakehead in the second sampling month in An Giang and Dong Thap provinces. *Vibrio* was isolated in the second and third sampling month with the clinical signs which were exothalmic, fins torn, white spot on the body surface and pale liver, kidne and spleen and sweeling bladder in visceral organs.

Bacterial colony grew on TSA at 28^{o}C in 24 hours with round, slight orange colour. Biochemical tests showed that Gram-negative, rod-shape, oxidase and catalase-positive, O/F-fermenting and O/129-sensitive. Based on morphological characteristics, biochemical tests and classification key of Barrow and Feltham (1993) *Vibrio* widentified from cultured snakehead was *Aeromonas* with frequency of apparence of 16.0%. *Vibrio* belong to the Family *Vibrionaceae*, Order *Vibrionales*. The exam of API 20E kit on biochemical properties identified two isolates of 24 and 27 were *Vibrio cholerae*, but some differences were demonstrated by API 20E kit on the other strains, which were classified to genus of *Vibrio.*

Vibrio harvegi and *V. samonicida* were recorded as a main pathogen damaging to fishes, bivalvia and crustacean (Toranzo and Barja, 1990; Sørum *et al.,* 1992). The clinical signs are small red spot on the fish body, removing of scales and forming of ulcerative lesion and fins torn or necrosis (Tolmasky *et al.,* 1995). A similarity was found in present study to the previous studies.

Pseudomonas

The study isolated a total of 11 *Pseudomonas* strains from cultured snakehead in An Giang and Dong Thap provinces. Bacterial strains were isolated in the third sampling month (fish weight was 280-320 g) and the sixth sampling month (fish weight was 850-1100 g). The clinical signs were noted with poor

swimming fish, spinning swim, imbalance with the head down, haemorrhage in both body sides. Moreover, swelling of liver, spleen and gallbladder were noted in abdominal cavity.
After 24 hours, bacterial colony grew rapidly on TSA at 28^{o}C with round, whitish, slightly convex. Biochemical tests indicated that Gram-negative, rod-shape, motile, oxidase and catalase-positive, O/F-nonfermenting. Based on morphological characteristics, biochemical tests and classification key of Barrow and Feltham (1993) bacterial strains isolated from cultured snakehead was *Pseudomonas* with frequency of apparence of 13.6%. *Pseudomonas* belong to the Family *Pseudomonadaceae*, Order *Pseudomonadales*. The exam of API 20E kit on biochemical properties identified two isolates of 75 and 81 were *Pseudomonas fluorescens*, but some differences were demonstrated by API 20E kit on the other strains, which were classified to genus of *Pseudomonas.*

According to Sindermann (1990) defined that *P. anguilliseptica*, *P. chlororaphus* caused haemorrhage signs in marine fish. Moreover, *P. flourescens* and *P. pseudoalcaligenes* were believed that mainly distributed and caused haemorrhage in freshwater fish (Ahne *et al*., 1982; Wiklund *et al.,* 1994). *Pseudomonas* was isolated in the cultured snakehead culture in An Giang and Dong Thap provinces, although the genus was isolated in other aquatic animals.

Streptococcus
Total of 12 *Streptococcus* strains were isolated from cultured snakehead in the only the second sampling month in An Giang and Dong Thap provinces. The fish samples weighted 580-820 g/fish (in the fifth sampling). The clinical signs were noted with abnormal swimming behavior, body swelling, scabrous scales and ulcerative lesion in the body (Fig. 16A). Moreover, necrosis of liver and kidney and normal spleen were regconized in abdominal cavity (Fig. 16B).

Bacterial colony grew quickly on TSA at 28^{o}C in 48 hours. The colonies were round, yellow, slightly convex (Fig. 16C). The results of biochemical and physiological tests demonstrated that Gram-positive, spherical-shape, non-motile (Fig. 16D), oxidase and catalase-negative, O/F-fermenting. Basing on morphological characteristics, biochemical tests and classification key of Barrow and Feltham (1993) bacterial strains isolated from cultured snakehead was *Streptococcus* with frequency of apparence of 14.8%. *Streptococcus* belong to the Family *Streptococcaceae*, Order *Lactobacillales*.

Streptococcus was firstly found in *Lates calcarifer* and *Mugil cephalus* with hemorrhagic signs (Plumb *et al*., 1974). Furthermore, *Streptococcus* probably caused haemorrhage in *Oreochromis niloticus*, *Pangasius bocourti* and *Cyprius carpio* (Robinson and Meyer, 1966). The present study showed the first regconization of *Streptococcus* in cultured snakehead culturing in An Giang and Dong Thap.

CONCLUSIONS

The main water quality parameters are successfully recorded in this study. The results of this study illustrated that temperature and pH value in snakehead cultured ponds in An Giang and Dong Thap provinces were not considerably varied. In the whole cultured period, the concentration of $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$ increased, while the concentration of $N\text{-}NO_3^-$ reduced in the posterior end.
The average age of snakehead farming households was 44.7±9.12. The education level was low, with 60% of households attainding primary school. On average, snakehead farming households had 9±4.4 years experiences in culturing snakehead in the Mekong delta. Improvement of snakehead culture skills was mostly from the gain of experiences and participation in trainings provided by provincial fisheries officers. Water quality management was applied by the majority of household (75%) such as the use of test kits. Most of the snakehead farming household (70%) complain parasite infection to their snakehead fish. Therefore, parasite infection is the key issue in snakehead farming in the Mekong Delta of Vietnam.

The potential pathogens, including parasites, fungi and bacteria infesting snakehead fingerlings and cultured snakehead in An Giang and Dong Thap provinces, Vietnam are successfully identified, classified and documented. Nine genera of parasites were identified from 142 samples of snakehead fingerlings in nursery ponds, including of *Dactylogyrus*, *Trichodina*, *Epistylis, Trianchoratus, Chilodonella*, *Proteocephalus*, *Spinitectus*, *Pallisentis*. *Trichodina* had the highest prevalence infection of 93.7%, while *Proteocephalus* and *Henneguya* had lowest prevalence infection of 3.52% and intensity of lowest (+). There were 23 genera of parasites identified from cultured snakehead sampled from growth-out ponds in An Giang and Dong Thap provinces. Of which, 6 new genera were found in cultured snakehead, comprising of *Henneguya, Chilodonella, Epistylis, Tripartiella, Gnathostoma* and *Capillaria*. *Gyrodactylus* was noted with the highest prevalence infection of 72.6% and the lowest was found in *Lamproglena* (0.7%).

Achlya sp. was isolated from snakehead fingerlings in nursery ponds in the first and second month of culture period. The frequency of apparence was 46% in the whole isolated fungi recorded. The optimum temperature for *Achlya* sp. VN1101 growth was 28^{o}C. The results demonstrated that *Achlya* sp. VN1101 was main pathogen causing cotton-like in snakehead fingerlings revealed by pathogenicity experiment. There were 4 genera of fungi isolated from cultured snakehead in grow-out ponds in An Giang and Dong Thap provinces, including *Acremonium* (frequency of apparence was 35.7%), *Geotrichum* (28.6%), *Achlya* (21.4%) and *Fusarium* (14.3%). The fungi were regconized in the first three months of culture period, and *Achlya* was only noted in the first month of the cultured period when the fish was 96.6-165 g in weight.

81 bacterial strains were indentified from unhealthy fish. Bacterial strains were grouped to 5 genera based on morphological and biochemical characteristics, comprising of *Aeromonas* (frequency of apparence of 38.3%), *Edwardsiella* (17.3%), *Vibrio* (16.0%), *Streptococcus* (14.%) and *Pseudomonas* (13.6%). Of which, *Edwardsiella* was only isolated in the second month of the cultured period when fish was 175.7-295.3 g in weight, and *Streptococcus* was also detected in the fifth cultured month when fish was 620.4-850 g in weight. Besides, *Aeromonas hydrophila* was believed as a primary pathogen to snakehead fingerlings revealed by pathogenicity experiment. The clinical signs were observed, consisting of haemorrhage in fish body and fins, red spot in ventral area, and scales damage and loss.

RECOMMENDATIONS

The implement sustainable snakehead aquaculture development in the Mekong Delta of Vietnam the following recommendations are proposed for policy and action plan development for snakehead disease and health management:

- At least eight kinds of chemicals have been used by snakehead farmers in the Mekong Delta for treating diseases, social, envrironmental and economical risk and impact assessment should be conducted to sustain this industry;
- Research into traditional or low environmental impact snakehead disease treatment by using local available plants or herbs;
- Research into how to control and treat diseases in snakehead and how to manage fish health better.
- Further research into snakehead virus diseases;
- Promote and implement awareness raising by producing and disseminating extension materials, for example snakehead fish disease cards to snakehead farmers;

- Train snakehead farmers and provincial fisheries extension officers on snakehead fish disease and health management;
- Knowledge, skills and information gained from this study on snakehead fish diseases should be transfer to colleagues in Cambodia;
- A national list of snakehead fish diseases should be development and legalized;
- Strengthen the national and sub-national capability in epidemiology, risk analysis, disease surveillance, contingency planning, and information management, reporting and communication related to the biosecurity of snakehead aquaculture industy;
- A trans-boundary framework for snakehead disease and health management between Vietnam and Cambodia should be established to prevent or reduce trans-boundary diseases caused by spread of snakehead pathogens, which have caused significant damage in recent years in Vietnam and Cambodia; and
- Encourage public and private sectors to invest on health management and biosecurity of snakehead aquaculture industry because of the social and economic importance of the snakead fishery and aquaculture sector.
-

ACKNOWLEDGMENTS

This study was funded by AquaFish CRSP. The authors would like to thank An Giang, Dong Thap and Can Though Rural Development and Agriculture Departments for their kind support and cooperation in data collection in the field.

REFERENCES

Ahne, W., W. Popp and R. Hoffmann, 1982. *Pseudomonas fluorescens* as a pathogen of tench (*Tinca tinca*). Bulletin of the European Association of Fish Pathologists, 4:56-57.

Alderman, D. J. and J. L. Polglase. 1985. *Fusarium tabacinum* (Beyma) Gams as a gill parasite in the crayfish, *Austropotamobius pallipes*. Journal of Fish Diseases. 8:249-252.

Angka, S.L., 1990. The Pathology of the walking catfish Clarias batrachus, infected intraperitoneally with Aeoromonas hydrophila Asian Fish S3. In Asian Fish Health Biblography and Abstracts I: Southeast Asia. Fish Health Section Asian Fisheries Society Manila, Philippines, 1992. 343-351.

Arthur, R. and B.Q. Te, 2006. Checklist of the Parasites of Fishes of the Viet Nam. FAO Fisheries Technical Paper No. 369/2. Rome, 2006, 140 pp.

Azevedo, C. and E. Matos, 1996. *Henneguya malabarica* sp. nov. (Myxozoa, Myxobolidae) in the Amazonian fish *Hoplias malabaricus*. Parasitol. Res. 82:222–224.

Barrow, G.I. and R.K.A. Feltham. 1993. Cowan and Steel's manual for the indentification of medical bacteria, third edition. Cambridge University press. Cambridge. 331 pp.

Bùi Quang Tề và Vũ Thị Tám, 2000. Những bệnh thường gặp của tôm cá và biện pháp phòng trị. Nhà xuất bản Nông Nghiệp. 79 trang.

Bùi Quang Tề, 2001. KST của một số loài cá nước ngọt ở Đồng bằng Sông Cửu Long và các giải pháp phòng trị chúng. Luận án Tiến sỹ Sinh học. Trường Đại học quốc gia Hà Nội.

Coker, W.C. 1923. The *Sarproleniacece* with notes on other water molds. The University of North Carolina Press. Chapel Hill. 201 pp.

de Hoog, G.S., J. Guarro, J. Gené and M.J. Figueras. 2000. Atlas of clinical fungi. 2nd edition. Centraalbureau voor schimmelculture. 1126p.

Duc, P.M. and K. Hatai, 2009. Pathogenicity of anamorphic fungi *Plectosporium oratosquillae* and *Acremonium* sp. to mantis shrimp, *Oratosquilla oratoria*. Fish Pathology. 44:81-85.

Farooqi, H.U., 1958. A new species of the genus Pallisentis from a fresh-water eel. Parasitenkunde, Bd. 18:457—464.

Furguson, H.W, J.E. Turnbull, A. Shinn, K. Thompson, T.T. Dung and M. Crumlish. 2001. Bacillary nercrosis in farmed *Pangasius hypophthalamus* (Sauvage) from the Mekong Delta, Viet Nam. Journal of Fish Diseases. 24:509-513.

Gams, W., H.A. van der Aa, A. J. van der Plaats-Niterink, R. A. Samson and J. A. Stalpers, 1980. CBS course of mycology. Centraalbureau voor Schimmelcultures Baarn (The Netherlands), Institute of the Royal Netherlands, Academy of Science and Letters, Amsterdam-Zuid. 109pp.

Hà Ký và Bùi Quang Tề, 2007. KST nước ngọt Việt Nam. Nhà xuất bản Khoa Học và Kỹ Thuật Hà Nội. 360 trang.

Hatai, K., D. Roza and T. Nakayama. 2000. Identification of lower fungi isolated from larvae of mangrove crab, *Scylla serrata*, in Indonesia. Mycoscience. 41:565-572.

Hatai, K., S. Kubota, N. Kida, S. Udagawa, 1986. *Fusarium oxysporum* in red sea beam, *Pagrus* sp. Journal Wildlife Diseases. 22:570–571.

Hawke, J.P. 1979. A bacterium associated with disease of pond culture channel catfish. Journal of the Fisheries Research Board of Canada. 36:1508-1512.

Johnson, T. W. 1956. The genus *Achlya* morpholology and taxonomy. The University of Michigan Press. 180 pp.

Kabata, Z., 1981. Copepoda (Crustacea) parasitic on fishes: problems and perspectives. Adv. Parasitol. 19:1-71.

Khoa, L.V., K. Hatai, and T. Aoki, 2004. *Fusarium incarnatum* isolated from black tiger shrimp *Penaeus monodon* Fabricius, with black gill disease cultured in Vietnam. Journal of Fish Diseases. 27:507-515.

Khoa, L.V. and K. Hatai, 2005. First case of *Fusarium oxysporum* infection in culture Kuruma Prawn *Penaeus japonicus* in Japan. Fish Pathology. 40:195-196.

Kino, H., H. Inaba, N.V. De, L.V. Chau, D.T. Son, H.T. Hao, N.D. Toan, L.D. Cong and M. Sano, 1998. Epidemiology of clonorchiasis in Ninh Binh Province, Vietnam. Southeast Asian J. Trop. Med. Publ. Health. 29:250-254.

Lê Xuân Sinh và Đỗ Minh Chung. 2009. Hiện trạng và những thách thức cho nghề nuôi cá lóc *(Channa micropeltes* và *Channa striatus)* ở ĐBSCL. Báo cáo trình bày tại Hội thảo kết thúc giai đoạn 1 – Dự án Cá Tạp - Khoa Thủy sản - ĐHCT, 8-12/20

Moravec, F., R. Aguilar and G. Salgado-Maldonado, 2001. Systematic status of *Capillaria patzcuarencis* Osorio-Sarabia, Perez-Ponce de Leon et Salgado-Maldonado, 1986 (Nematoda, Capillariidae) from fresh water fishes in Mexico. Acta Parasitologica. 46(1):8-11.

Nguyễn Thị Diệp Thúy. 2010. Phân tích một số chỉ tiêu kinh tế - kỹ thuật của các mô hình nuôi cá lóc ở Đồng bằng sông Cửu Long. Luận văn tốt nghiệp cao học. Khoa Thủy Sản - Trường Đại Học Cần Thơ.

Nguyễn Thị Thu Hằng, Đặng Thụy Mai Thy, Nguyễn Thanh Phương và Đặng Hoàng Oanh, 2008. Khảo sát sự nhiễm KST trên cá tra (*Pangasinodon hypophthalmus*) nuôi thâm canh ở tỉnh An Giang. Tạp chí khoa học. Đại Học Cần Thơ. 2008(1):204– 212.

Nguyễn Văn Thường, 2004. Tổng quan về thành phần loài và phân bố của cá họ *Channidae*. Tạp chí khoa học Đại Học Cần Thơ, chuyên ngành thủy sản. (2). Trang 14-24.

Pin, N., 1997. *Proteocephalus sinocyclocheili* n. Sp. (Cestoda: Proteocephalidae) in a fish *Sinocyclocheilus grahami tingi* fang from Yunnan, China. Chine,Se Journal of Oceanology and Limnology. 15:61-64.

Plumb, J.A. and D.J. Sanchez, 1983. Susceptibility of 5 species of fish to *Edwardsiella ictaluri*. Journal of Fish Diseases. 6:261-266.

Plumb, J.A., J.H. Schachte, J.L. Gaines, W. Peltier and B. Carroll, 1974. *Streptococcus* sp. from marine fishes along the Alabama and Northwest Florida coast of the Gulf of Mexico. Transacions of the American Fisheries Society. 2:358-361.

Rahman, M.H., S. Yesmin, M. Afzal Hussain, A.R. Khan, F. Pervin and M.A. Hossain, 2004. *Aeromonas hydrophila* Infection in Fish of Swamps in Bangladesh. Pakistan Journal of Biological Sciences. 7(3):409-411.

Reda, A.S.E., 2011. A Review of some Ecto-and Endo Protozoan Parasites Infecting Sarotherodon Galilaeus and Tilapia Zillii from Damietta Branch of River Nile, Egypt. Journal of American Science. 7(3):362-373.

Rhoobunjongde, W., K. Hatai, S. Wada and S. Kubota, 1991. *Fusarium moniliforme* isolated from gills of kuruma prawn *Penaeus japonicus* with black gill disease. Nippon Siusan Gakkaishi. 57:629-635.

Robinson, J.A. and F.P. Meyer, 1966. *Streptococcus* fish pathogen. Journal of Bacteriology 92. 512 p.

Sarowar, M.N., M.Z.H. Jewel, M.A. Sayeed and M.F.A. Mollah, 2010. Impacts of different diets on growth and survival of *Channa striatus* fry. Int. J. BioRes. 1(3):08-12.

Scott, W. W. 1961. A monograph of the genus *Aphanomyces*. Technical Bullentin 151. Blacksburg, Virginia. 94 pp.

Seymour, R. L. 1970. The genus *Saprolegnia*. The University of Pittsburgh, Pittsburgh, Pennsyvania. Germany. 224 pp.

Sindermann, C.J., 1990. Principal Diseases of Marine Fish and Shellfish, Vol 1: Diseases of Marine Fish, 2nd edn. Academic Press, New York.

Sitja-Bobadilla, P. Alvarez and P. Pellitero, 1993. Ultrastructural and cytochemical observations on the sporogenesis of *Sphaerospora testicularis* (Protozoa: Myxosporea) from Mediterranean sea bass, Dicentrachus labrax. European Journal of Protistology. 29 (2):219-229.

Sørum, H., M. Roberts and J.H. Crosa, 1992. Identification and cloning of a tetra-cycline resistance gence from the fish pathogen *Vibrio salmonicida*. Antimicrobial Agents and Chemotherapy. 36:611-615.

Tanasomwan, V. and K. Saitanu. 1979. Ulcer disease in strided catfish (*Pangasius pangasius*). Animal. In Asian Fish Health Biblography and Abstracts I: Southeast Asia. Fish Health Section Asian Fisheries Society Manila, Philippines, 1992. Dis 2:131-133.

Thien, P.C., A. Dalsgard, N.T. Nguyen, A. Olsen and K.D. Murell, 2009. Prevalence of zoonotic Trematoda parasites in fish fry and juveniles in fish farms of Mekong Delta, Việt Nam. Aquaculture. 295(1):1-5.

Tolmasky, M.E., L.A Actis and J.H. Crosa, 1995. Ahistidine decarboxylase gene encoded by the *Vibrio anguillarum* plasmid pJM1 is essential for virulence: histamne is a precursor in the biosynthesis of anguibactin. Molecular Microbiology. 15:87-95.

Toranzo, A.E. and J.L. Barja, 1990. Review of the taxonomy and seroepizootiology of *Vibrio anguillarum*, with special reference to acquaculture in the northwest of Spain. Diseases of Aquatic Organisms. 9:73-82.

Trần Ngọc Tuấn. 2010. Phân lập và định danh nấm trên cá rô đồng (*Anabas testudineus*) nuôi thâm canh. Luận văn tốt nghiệp cao học. Khoa Thủy Sản - Trường Đại Học Cần Thơ.

Trương Quốc Phú. 2006. Quản lý chất lượng nước nuôi trồng thủy sản. Khoa Thủy Sản. Trường Đại Học Cần Thơ. 201 trang.

Wiklund, T. and L. Lonnstrom, 1994. Occurrence of *Pseudomonas anguilliseptica* in Finnish fish farms during 1986 – 1991. Aquaculture 126:211-217.

Yunia A.T., H.W. Palm and T. Walter, 2007. Crustacean fish parasites from Segara Anakan Lagoon, Java, Indonesia. Parasitology Research, 100(6):1193-1204.

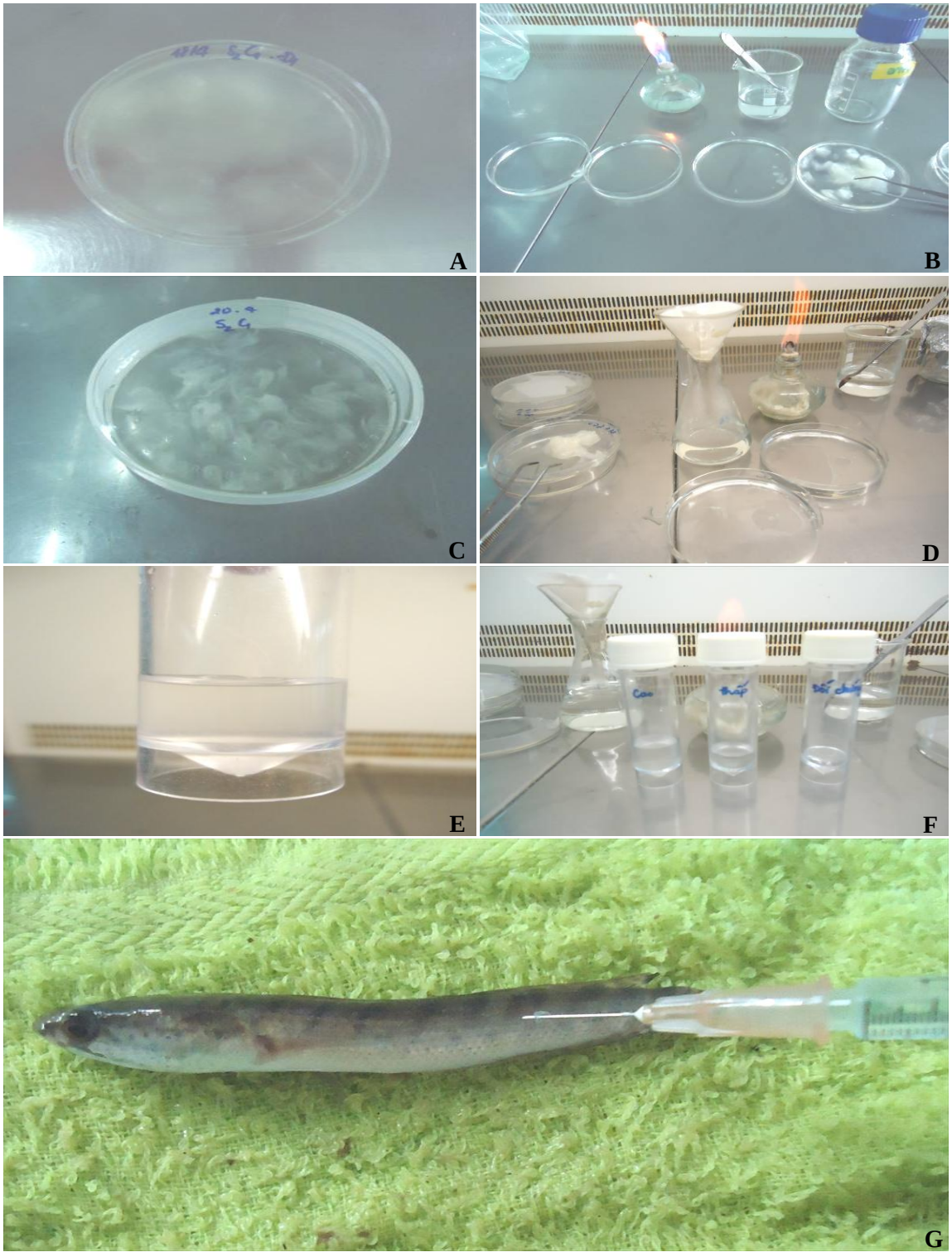


Figure 7: Experimentally infected with *Achlya* sp. VN1101: A) Fungal isolate *Achlya* sp. VN1101 in broth GY; B) washed in tap water in 3 times; C) Incubation in tap water; D) conidia fillter; E) Conidia solution; F) Adjusment conidia for experiment infection; G) Intramuscularly infected with 0.1 mL conidia.

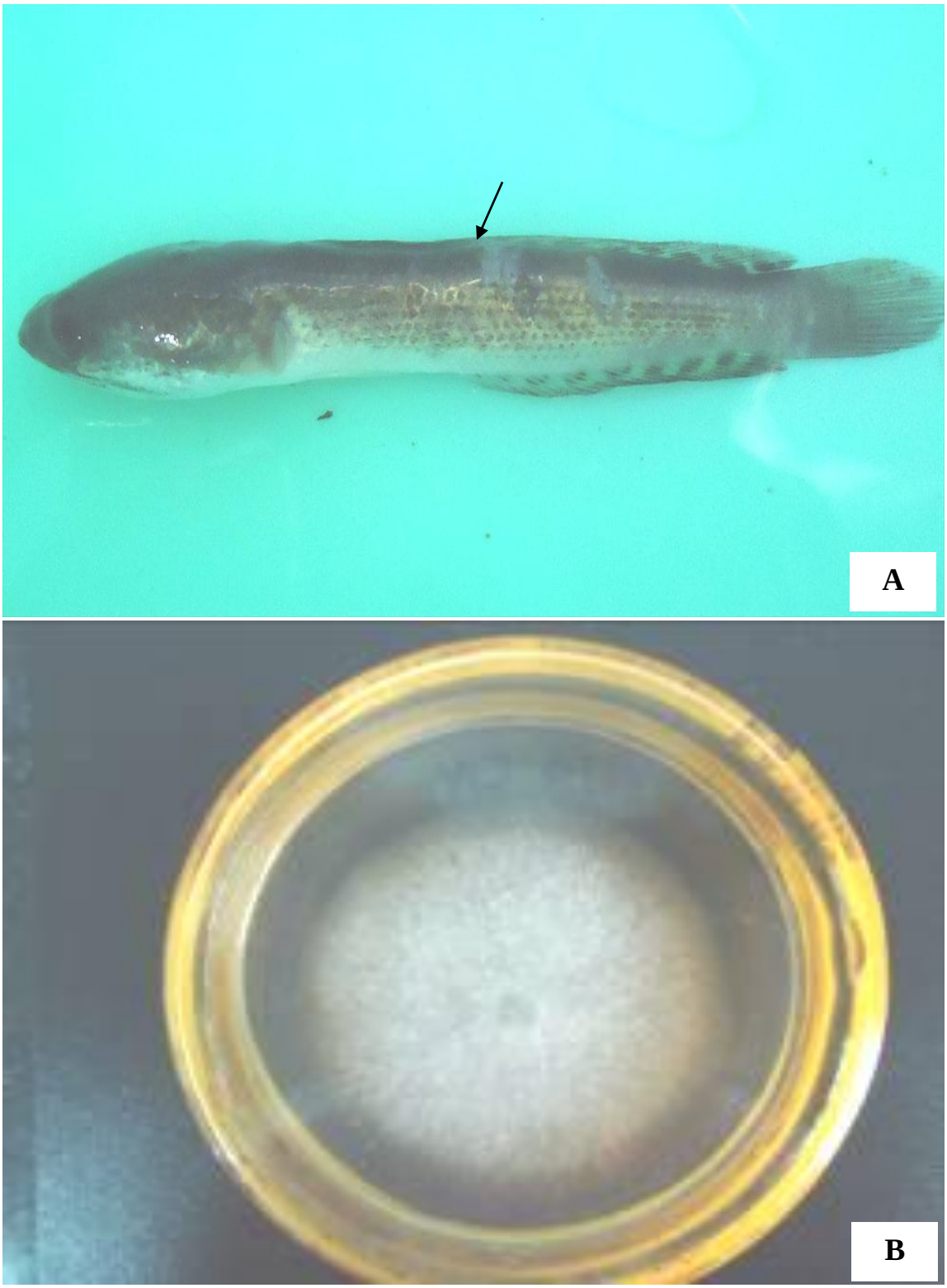


Figure 8: Experimentally infected with *Achlya* sp. VN1101: A) Clinical sign as siminar as fungal infection in pond (arrow); B) Reisolated fungal *Achlya* sp. VN1101 on GYA at 28^{o}C, after 3 days incubation.

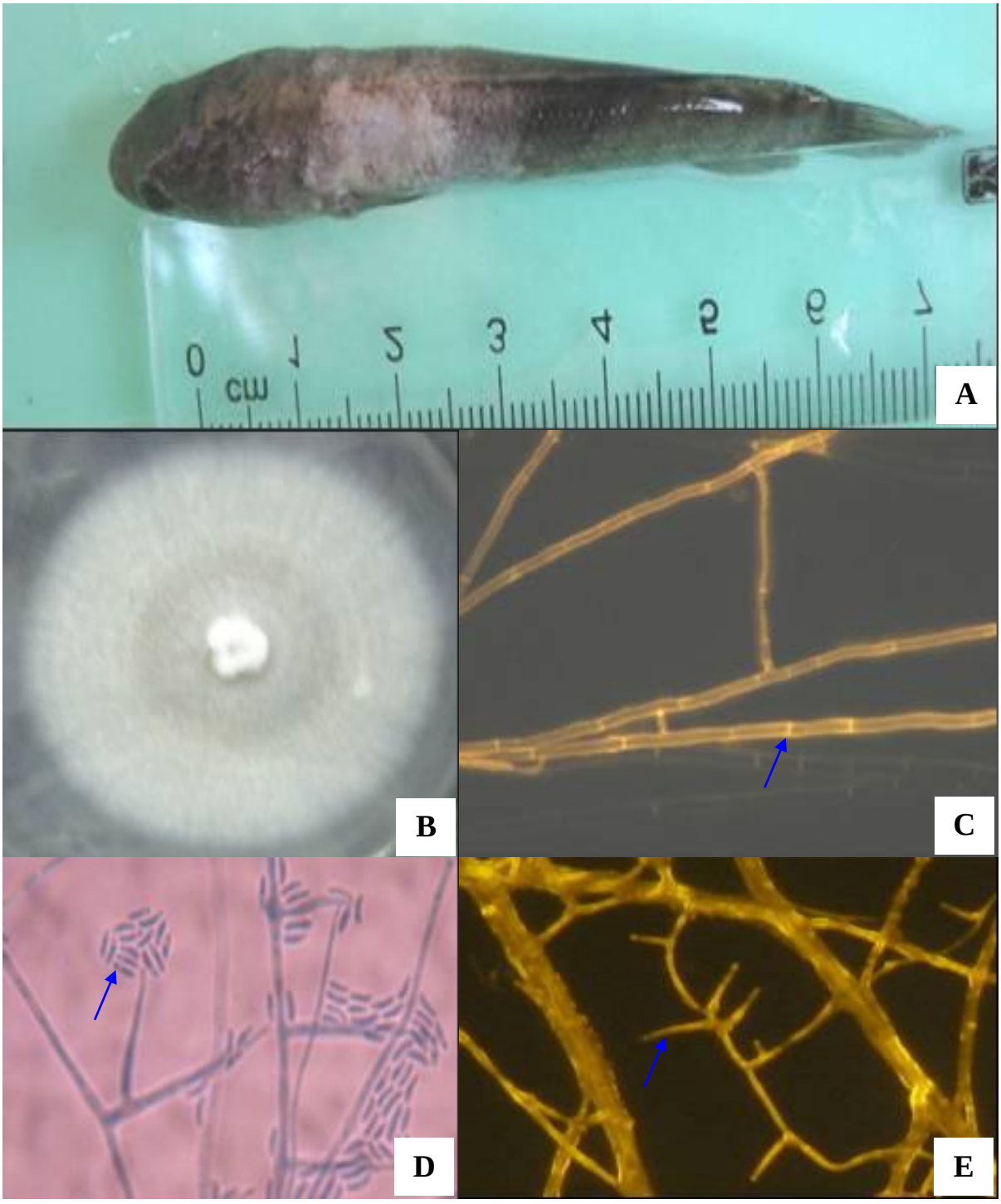


Figure. 9: Fungal ***Acremonium*** infected on snakehead fish in growth out pond: A) White skin with excess mucus; B) Colony of *Acremonium*; C) fungal with septate (1000x); D) Conidia (arrow, 1000x); E) Conidiophore (arrow, 1000x).

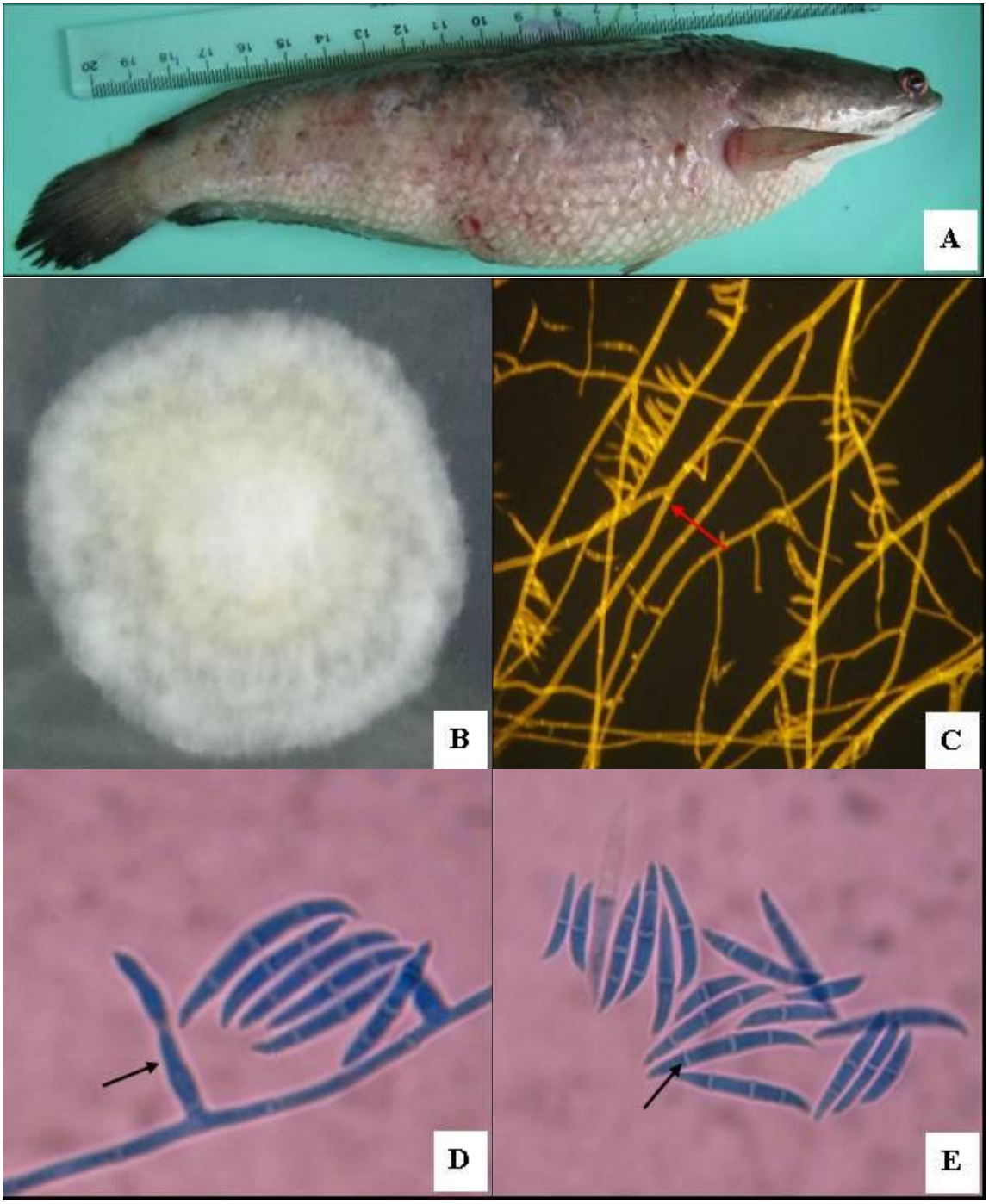


Figure. 10: Fungal *Fusarium* infected on snakehead fish in growth out pond: A) Concentrated mucus and rough scales; B) Colony *Fusarium* isolate on GYA at 28^{o}C after 6 days incubation; C) Fungal hyphae with septate (arrow, 1000x); D) Conidiophore (arrow) (stained with cotton-blue, 1000x); E) Conidia with septate (arrow, 1000x).

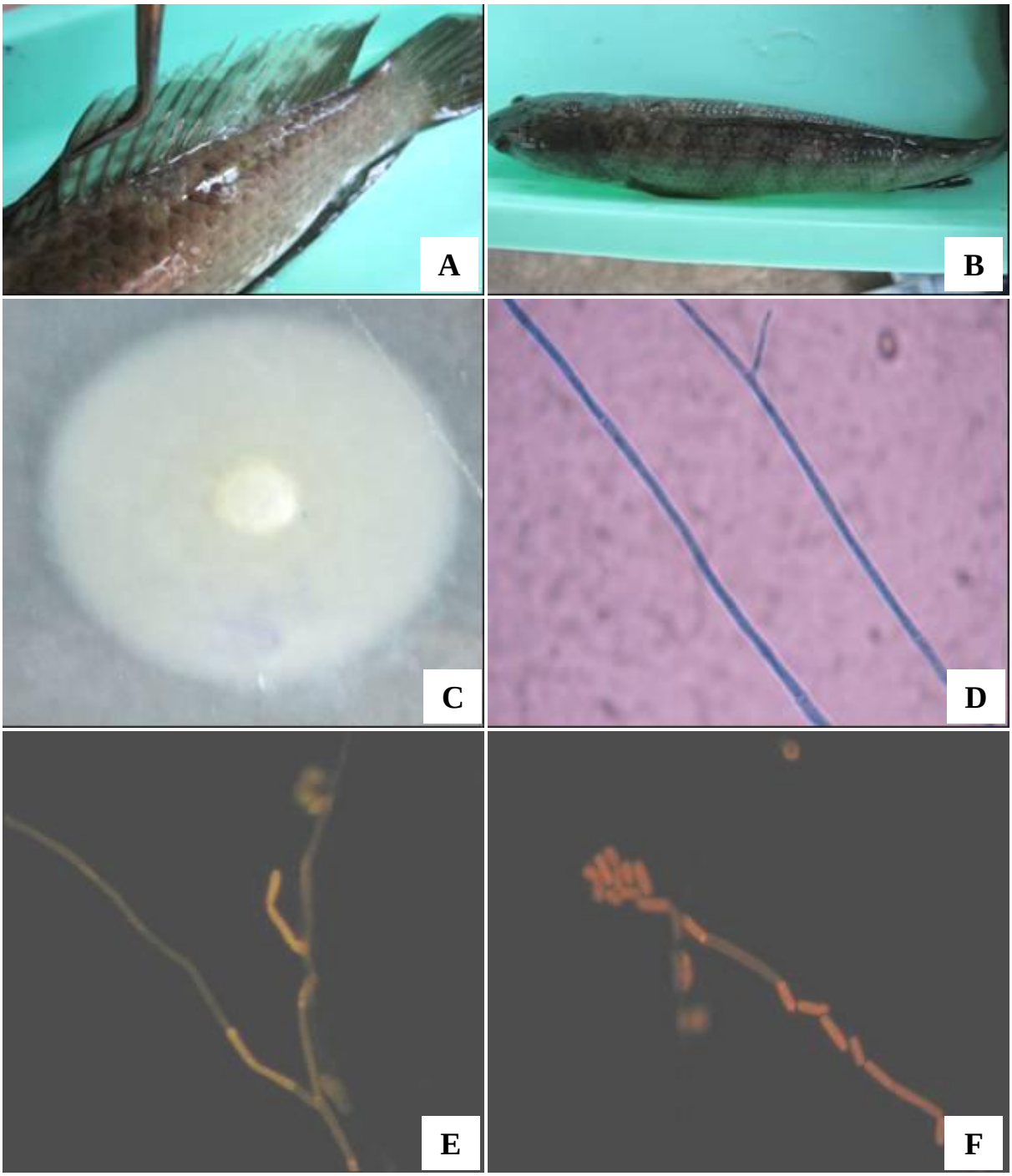


Figure 11: Fungus *Geotrichum* infected on snakehead fish in growth out pond: A) access mucus and rough scales; B) white spot; C) Colony *Geotrichum* isolate on GYA at 28°C after 6 days incubation; D) Fungal hyphae with septate (arrow, 1000x); E) Conidiophore (arrow) (stained with cotton-blue, 1000x); E) Conidia with septate (arrow, 1000x).

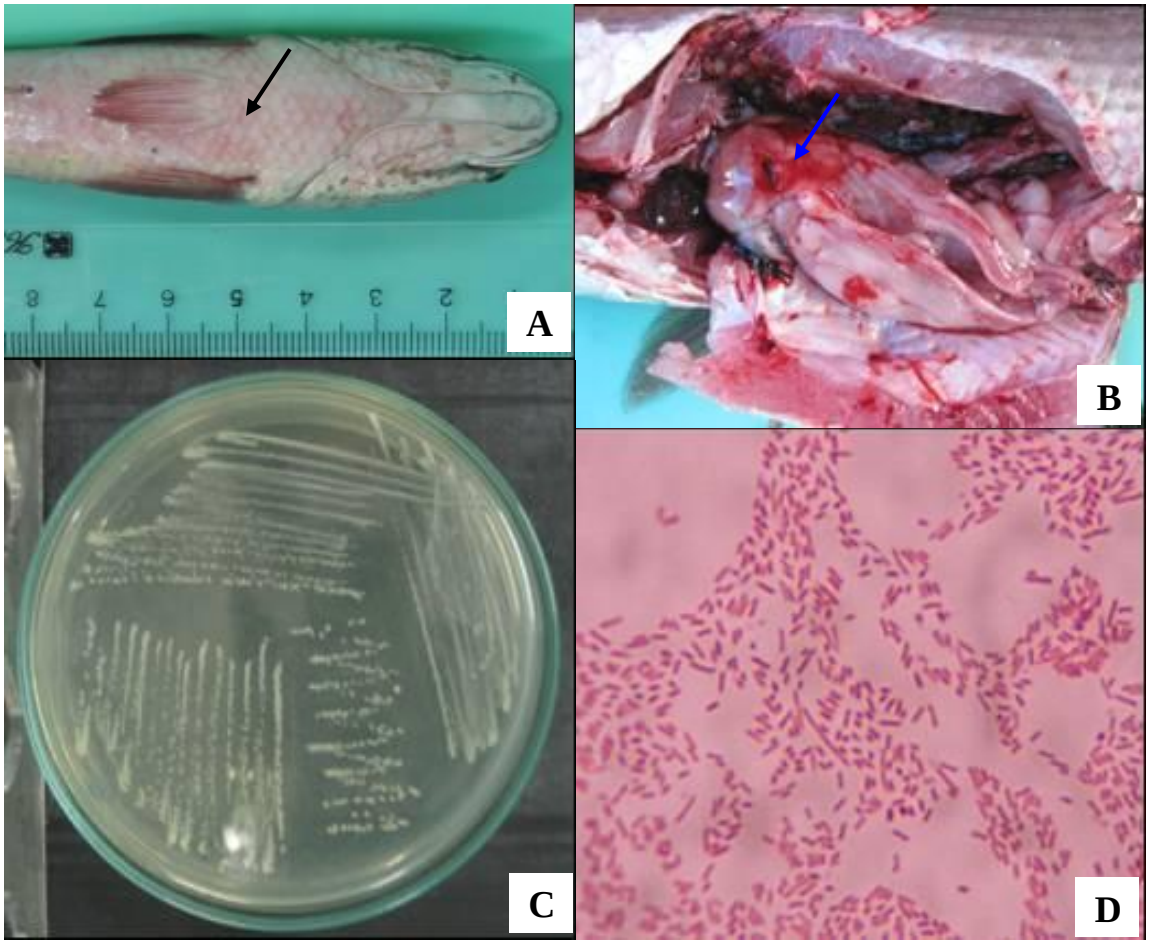


Figure 12: Bacteria *Aeromonas* isolated from snakehead fish disease in growth out pond: A) fish with red spot on body (arrow); B) Liver with red spot (arrow); C) Colony of isolate *Aeromonas* on TSA; D) Short rod shape and Gram negative (1000x).

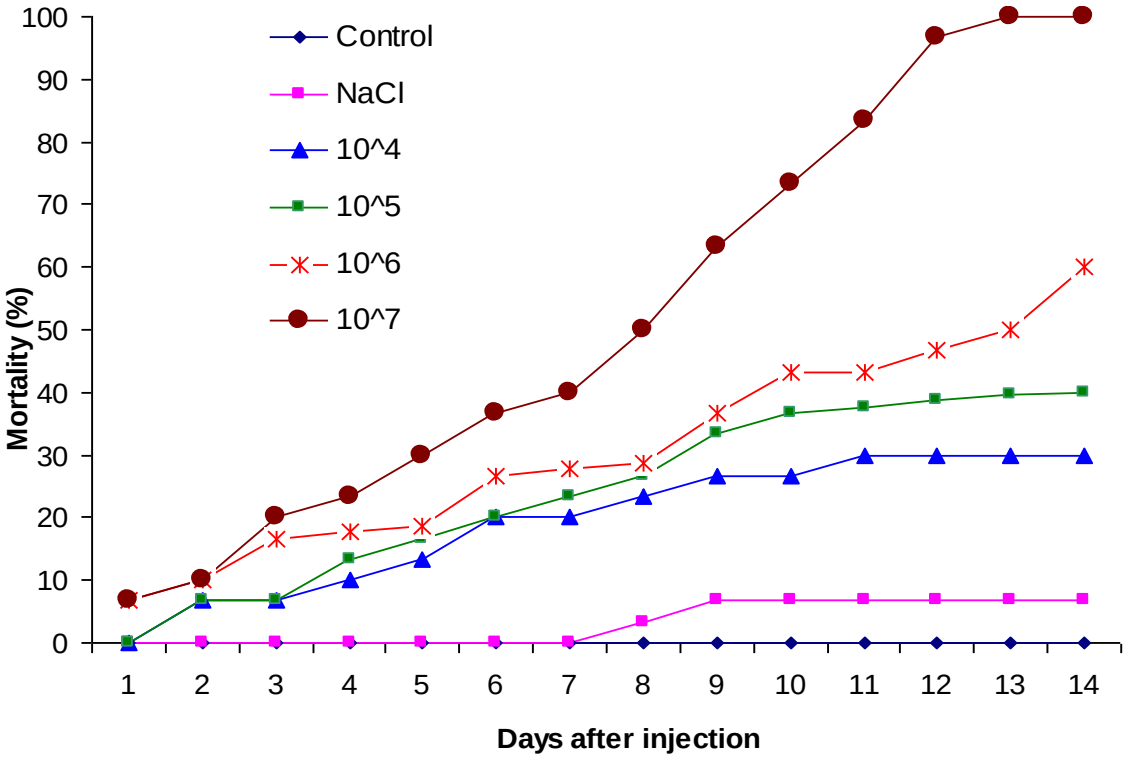
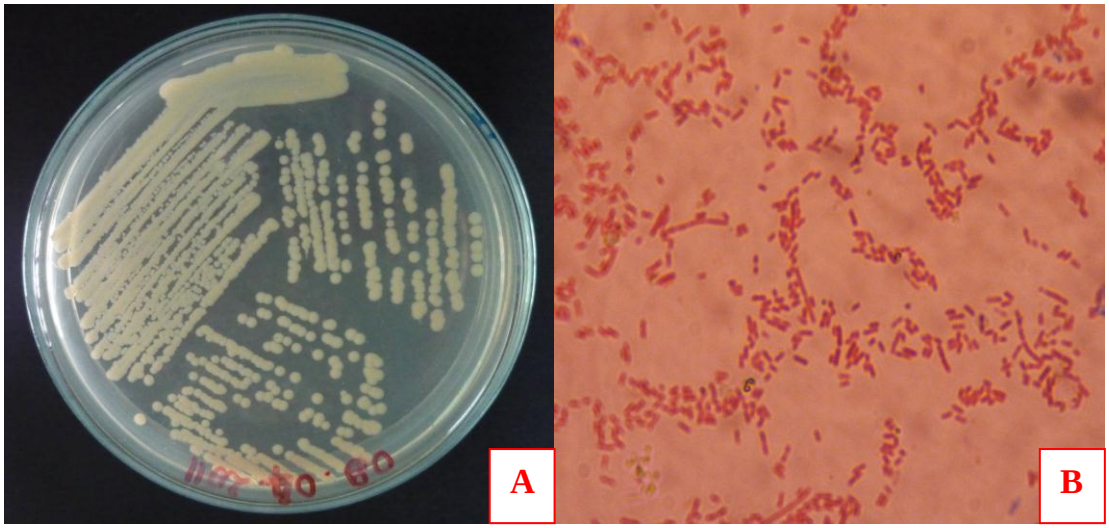


Figure 13: The cumulative mortality of snakehead fish experimentally injected with *Aeromonas hydrophila* CT1107.



B

Figure 14: Bacterial isolate *Aeromonas hydrophila* CT1107 reisolated from experimental injected fish: A) *Aeromonas hydrophila* on TSA; B) Shape of *Aeromonas hydrophia* Gram negative, rod (1000x).

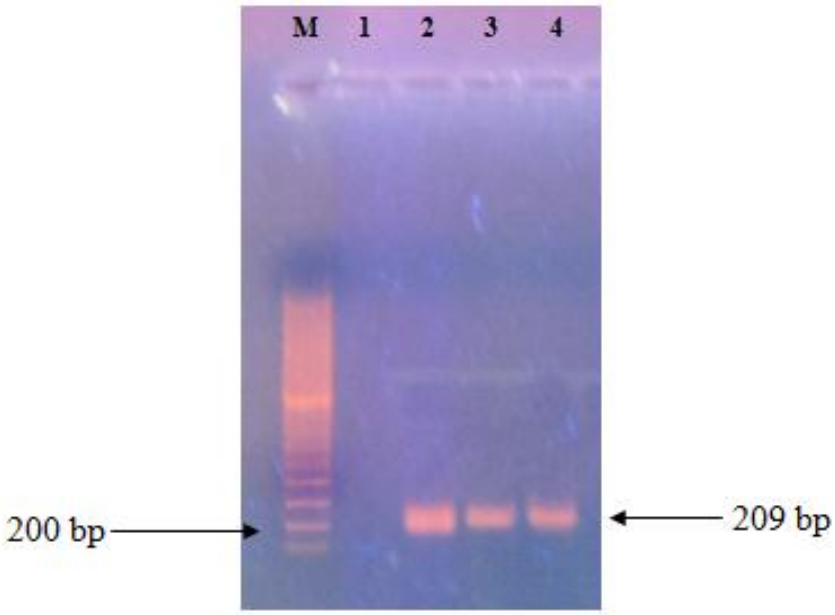


Figure 15: PCR for detected *Aeromonas hydrophila*: Band M: Maker; Band 1: Negative control (-) *Aeromonas hydrophila*; Band 2: Possitive control (+) *Aeromonas hydrophila*; Band 3: samples isolate CT1107, Band 4: samples isolate CT1109.

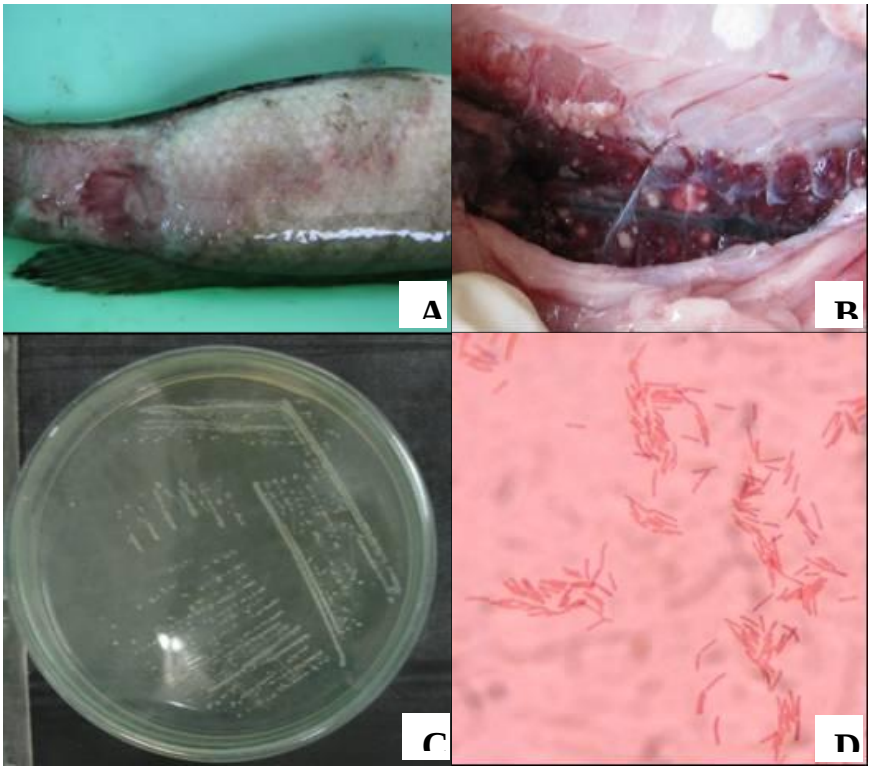


Figure 16: Bacteria *Edwardsiella* isolated from snakehead fish disease in growth out pond: A) fish with ulcerative on body; B) Kidney with white nodules; C) Colony of isolate *Edwardsiella* on TSA; D) Rod shape and Gram negative (1000x).

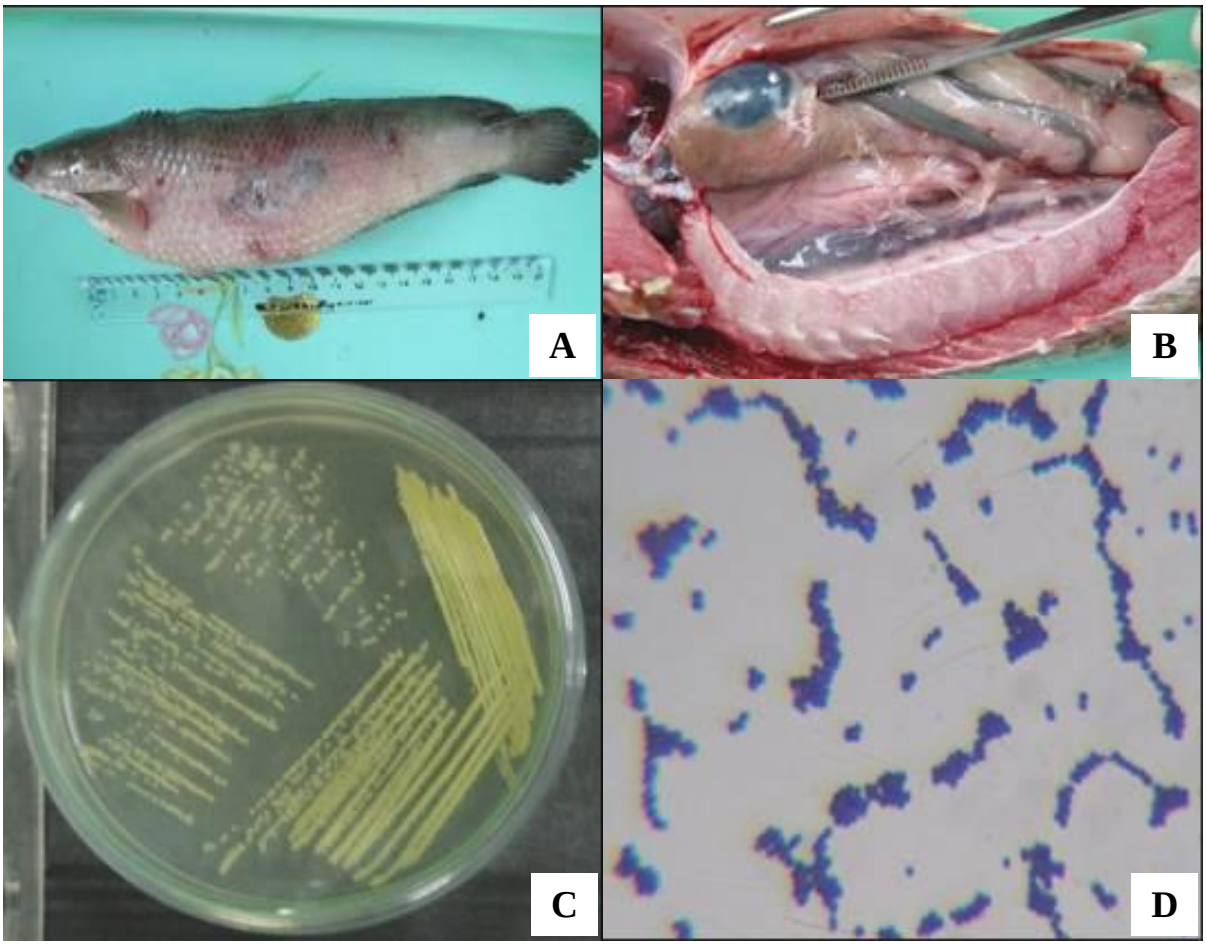


Figure 17: Bacteria *Streptococcus* isolated from snakehead fish disease in growth out pond: A) fish with ulcerative on body; B) Kidney dark red; C) Colony of isolate *Streptococcus* on TSA; D) Sphere shape and Gram possitive (1000x).

Table 1: Water quality parameters on snakehead fish cultured pond in An Giang and Dong Thap Province in 2010

Parameters	Location (Province)	Samples site	Cultured period (month)					
			1	2	3	4	5	6
Temperature (^oC)	An Giang	Pond	30.3±0.58	31.7±0.58	28.3±0.58	32.7±0.00	32.0±0.00	30.3±0.29
		inlet	30.3±0.58	31.0±1.00	29.0±0.00	32.0±0.00	32.3±0.58	30.0±0.00
	Dong Thap	Pond	31.3±0,58	30.3±0.29	26.7±0.58	32.5±0.50	30.3±0.58	31.0±0.00
		inlet	30.0±0.00	31.0±0.00	29.7±0.58	31.7±0.58	31.0±0.00	30.7±0.58
pH	An Giang	Pond	7.48±0.05	7.65±0.05	7.25±0.09	7.95±0.57	7.81±0.52	7.80±0.25
		inlet	6.93±0.09	7.49±0.21	7.19±0.15	7.34±0.19	7.11±0.03	7.25±0.07
	Dong Thap	Pond	7.80±0.53	7.35±0.04	7.32±0.09	7.63±0.10	7.33±0.04	7.48±0.09
		inlet	7.06±0.06	7.10±0.06	7.06±0.06	7.08±0.04	7.06±0.04	7.11±0.09
DO (ppm)	An Giang	Pond	5.18±0.11	5.10±0.23	4.91±0.89	4.58±1.79	4.38±1.35	4.11±0.21
		inlet	5.20±0.02	5.20±0.14	4.95±1.18	4.65±0.27	4.43±0.67	4.12±1.11
	Dong Thap	Pond	4.86±1.01	4.35±0.32	4.68±0.20	4.43±0.76	4.57±0.34	4.29±0.71
		inlet	5.15±0.30	4.56±0.91	4.87±0.19	4.54±0.37	4.76±0.93	4.36±0.47
$N\text{-}NH_4^+$ (ppm)	An Giang	Pond	1.25±0.98	1.75±0.51	1.39±0.11	1.53±0.13	1.58±0.22	1.84±0.33
		inlet	0.04±0.02	0.11±0.02	0.08±0.04	0.41±0.52	0.07±0.05	0.25±0.18
	Dong Thap	Pond	1.28±0.96	1.59±0.56	1.43±0.31	1.64±1.35	1.79±0.94	1.95±0.78
		inlet	0.03±0.01	0.05±0.04	0.02±0.01	0.03±0.00	0.05±0.04	0.08±0.01
$N\text{-}NO_2^-$ (ppm)	An Giang	Pond	0.066±0.013	0.083±0.051	0.225±0.028	0.304±0.057	0.321±0.061	0.329±0.216
		inlet	0.010±0.003	0.023±0.010	0.057±0.046	0.035±0.032	0.011±0.007	0.061±0.038
	Dong Thap	Pond	0.040±0.021	0.056±0.007	0.075±0.010	0.131±0.063	0.144±0.090	0.209±0.162
		inlet	0.020±0.002	0.031±0.018	0.025±0.005	0.032±0.010	0.023±0.016	0.042±0.038
$N\text{-}NO_3^-$ (ppm)	An Giang	Pond	0.434±0.348	1.735±0.532	1.196±0.490	0.472±0.160	0.520±0.233	0.470±0.278
		inlet	0.066±0.048	0.163±0.032	0.093±0.044	0.033±0.034	0.044±0.027	0.104±0.068
	Dong Thap	Pond	1.066±0.352	1.229±0.387	0.355±0.072	0.301±0.089	0.301±0.089	0.266±0.050
		inlet	0.099±0.007	0.224±0.053	0.081±0.021	0.110±0.010	0.117±0.048	0.072±0.054

Table 2: Water quality parameters on snakehead fish cultured pond in Co Do District. Can Tho Province in 2010-2011

Parameters	Samples site	Cultured period (month)		
		2	3	4
Temperature	Pond	28.05±0.75	28.80±0.14	28.65±0.49
(^oC)	inlet	27.3	28.5	27.7
pH	Pond	8.18±0.09	8.39±0.09	8.75±0.16
	inlet	8.36	8.43	8.78
DO	Pond	4.81±1.21	4.68±0.29	4.51±0.92
(ppm)	inlet	5.12	4.93	4.66
$N\text{-}NH_4^+$	Pond	1.54±0.06	1.56±0.07	1.59±0.08
(ppm)	inlet	0.09	1.26	1.62
$N\text{-}NO_2^-$	Pond	0.03±0.01	0.07±0.00	0.08±0.01
(ppm)	inlet	0.12	0.14	0.27
$N\text{-}NO_3^-$	Pond	0.04±0.01	0.28±0.22	0.53±0.01
(ppm)	inlet	0.07	0.06	0.07

Table 3: Water quality parameters on snakehead fish fingerling cultured pond in Tam Nong District. Dong Thap Province in 2011

Parameters	Location (Province)	Samples site	Nursing period (week)	
			2	3
Temperature(^{o}C)	Dong Thap	Pond	31.83±0.56	31.55±0.26
		inlet	31.87±0.58	31.60±0.46
pH	Dong Thap	Pond	8.30±0.32	8.48±0.19
		inlet	8.43±0.45	8.10±0.26
DO (ppm)	Dong Thap	Pond	4.52±0.12	4.23±0.97
		inlet	5.18±1.02	4.97±0.56
$N\text{-}NH_4^+$ (ppm)	Dong Thap	Pond	1.16±0.31	2.27±1.14
		inlet	0.63±0.23	1.21±0.17
$N\text{-}NO_2^-$ (ppm)	Dong Thap	Pond	0.09±0.05	0.04±0.02
		inlet	0.09±0.03	0.05±0.01
$N\text{-}NO_3^-$ (ppm)	Dong Thap	Pond	0.31±0.18	0.31±0.24
		inlet	0.27±0.04	0.26±0.16

Table 4: Parasitic incedence on snakehead fish in growth out pond in An Giang and Dong Thap Provinces in 2010

No.	Parasites found	Site infected	Intensity of infection	Infected rate (%)
1	*Trichodina*	Skin, gill	1-25 ind/observation	93.66
2	*Epistylis*	Skin, gill	2-39 ind/observation	71.83
3	*Chilodonella*	Skin, gill	1-7 ind/observation	13.38
4	*Gyrodactylus*	Gill	1-4 ind/lame	12.68
5	*Trianchoratus*	Gill	1-3 ind/lame	5.63
6	*Proteocephalus*	Intestine	1-5 ind/lame	3.52
7	*Spinitectus*	Stomach	1-15 ind/stomach	19.01
8	*Pallisentis*	Stomach	2-11 ind/intestine	16.9
9	*Henneguya*	Gill	1-5 ind/observation	3.52

Table 5: Parasites found on fish based on monthly cultured by Provinces in 2010

No	Parasites found	Site infected	An Giang Province						Dong Thap Province					
			Cultured period (Mon)						Cultured period (Mon)					
			1	2	3	4	5	6	1	2	3	4	5	6
1	*Trichodina*	Skin	+		+					+				
2	*Apiosoma*		+	+					+		+			
3	*Myxobolus*		+	+		+		+		+		+	+	
4	*Epistylis*			+	+	+			+					
5	*Gyrodactylus*		+	+	+	+	+		+	+	+	+		+
6	*Tripartiella*		+			+			+	+				
7	*Lernaea*		+			+					+			
8	*Argulus*				+	+					+	+		
9	*Henneguya*	Gill	+		+					+				
10	*Chilodonella*			+							+			
11	*Trichodina*		+		+		+				+			
12	*Trianchoratus*			+								+		
13	*Lamproglena*				+									
14	*Ergasilus*			+						+				
15	*Gyrodactylus*		+	+	+				+	+	+	+	+	
16	*Haplorchis*	Fin		+										
17	*Clonorchis*				+									
18	*Exochis*					+								
19	*Polyonchobothrium*	Intestine			+					+				
20	*Proteocephalus*			+							+			
21	*Neocamallanus*		+	+	+					+		+	+	
22	*Pallisentis*		+	+	+		+			+		+		+
23	*Spinitectus*	Stomach	+	+	+	+		+		+	+			+
24	*Neocamallanus*			+	+						+	+	+	
25	*Gnathostoma*					+								
26	*Capillaria*			+										
27	*Neocamallanus*	SB											+	

Note: SB: Swim Bladde; (+): Parasite present

Table 6: Fungus infected on snakehead fish in growth out pond in An Giang and Dong Thap Provinces in 2010

No.	Fungus	An Giang Province						Dong Thap Province					
		Cultured period (Mon)						Cultured period (Mon)					
		1	2	3	4	5	6	1	2	3	4	5	6
1	*Achlya*	+						+					
2	*Fusarium*		+	+					+				
3	*Acremonium*		+						+	+			
4	*Geotrichum*		+	+					+	+			

(+)*: Fungus present*

Table 7: Bacteria isolated from snakehead fish in growth out pond in An Giang and Dong Thap Provinces in 2010

No.	Bacteria	An Giang Province						Dong Thap Province					
		Cultured period (Mon)						Cultured period (Mon)					
		1	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
1	*Pseudomonas*			+			+			+			+
2	*Aeromonas*	+	+	+	+	+	+	+	+	+	+	+	
3	*Edwardsiella*		+						+				
4	*Vibrio*		+	+					+	+			
5	*Streptococcus*					+						+	

(+): Bacteria present

Table 8: Biochemical characteristics of bacterial isolates from snakehead fish diseases in growth out pond in 2010-2011

Bacterial isolate Namely	Gram	Motility	Oxidase	Catalase	O/F	O/129
DT1012*	-	+	+	+	+/+	-
CT1101	-	+	+	+	+/+	-
CT1102	-	+	+	+	+/+	-
CT1103	-	+	+	+	+/+	-
CT1104	-	+	+	+	+/+	-
CT1105	-	+	+	+	+/+	-
CT1106	-	+	+	+	+/+	-
CT1107	-	+	+	+	+/+	-
CT1108	-	+	+	+	+/+	-
CT1109	-	+	+	+	+/+	-
CT1110	-	+	+	+	+/+	-
CT1111	-	+	+	+	+/+	-
CT1112	-	+	+	+	+/+	-

** Bacterial isolate for experimental injection*

DT: Dong Thap; CT: Can Tho

Induced Spawning and Larval Rearing of the "Chame" *Dormitator latifrons* in Laboratory Conditions

Indigenous Species Development/Experiment/09IND03UH

Gustavo A. Rodriguez Montes de Oca, Jose Cristobal Román Reyes, Eva Medina Hernandez, Jeniffer Velazquez Sandoval, Vanesa Lopez, Antonio Jiménez and Enrique Nuño
Universidad Autónoma de Sinaloa
Mazatlán, Sinaloa, México

Maria Haws and Armando Garcia
University of Hawai'i at Hilo
Hilo, Hawai'i, USA

Konrad Dabrowski
Ohio State University
Columbus Ohio, USA

Maria Celia Portella and Natalia de Jesus Leitão
Universidade Estadual Paulista
Jaboticabal, Brazil

ABSTRACT

The proposed research is focused on the production of chame (Pacific Fat Sleeper) juveniles for aquaculture and research purposes. Spawning was achieved using several delivery techniques for LHRHa. A series of experiments over two years have been conducted, during year 1 (2010) a trial was conducted using recently caught broodstock and for the year 2 (2011) a similar trial was carried out using breeders both males and females, with a whole year in captivity with the following treatments: control group (0.9% saline solution), Desgly^{10}-Ala^{6} LHRHa, Ovaprim® or a single implant of 75 µg Ovaplant®. Spawning results showed 80-100% success within 24h-48h for the Ovaplant group, 25-50% for LHRHa but 0% for Ovaprim within 48-72h for both years. Obtained data describes as oocyte size as 300 µm and a relative fecundity of 50,000 to 80,000 cells per gram. All LHRHa delivery treatments were effective to induce spermiation in volumes from 0.5 to 10 ml per male; however several males released sperm naturally up to 1 ml throughout the reproductive season. Data indicates that sperm activation time is close to 4 minutes, and overall concentration is within the range of 1 to 2X10^{9} cells per milliliter. Increased sperm motility is achieved after predilution on a 1:10-1:40 ratio in Ringer´s solution. As optimal salinity values, both for fertilization and egg incubation, our results indicate that there is no sperm activation above 5‰ of salinity. Data was recorded for optimal incubation salinity, as initially no hatching was observed above 15‰ salinity, although fertilized eggs can be transferred directly to salinities between 0-12‰ with no negative impact on hatching rate, total length (µm) yolk sack diameter and lipid drop size among salinity treatments. Extensive data has been recorded on incubation time at 26-28°C as 14 hours, hatching size of larvae (1-1.4 mm), time for yolk absorption (26-32 hours post fertilization hpf) and eye and mouth appearance time (30-40 hpf). Several feeding trials were conducted to initiate exogenous feeding; during year 1, feeding trails included live fresh water microalgae, dry powered microalgae, yeast and probiotics, and filtrated water from rotifer and tilapia rearing tanks and biofloc either as a single feed or a combination of 2 or 3 feeding treatments at a larval density

of 0.25-0.5 larvae per ml. For year two, we conducted a series of feeding trial using enriched rotifers or dry feeds, either at 0‰ or daily increments of 1.5‰ or fixed salinities within 2-8 ‰. Larvae from all trials on year one survived for only 6 days post fertilization at rearing temperature, with 100% mortality afterwards. However, last results for year 2 indicate that the use of either artificial plankton or an artemia substitute (2-8‰ rearing salinity) had values within 20-50% survival by day 6; although no survival was recorded after utilization of enriched rotifers. As preliminary conclusion, there is an apparent feeding on organic particulate matter for early larval stages as digestive track develops and matures for later consumption of live feeds such as rotifers and other zooplankton organisms, fact to be later validated as continued experiments for future research.

INTRODUCTION

Chame (*Dormitator latifrons*) (Fig. 1) also known as puyeque, popoyote, chalaco, Fat sleeper among other is a species of special interest for aquaculture. At present, medium-scale commercial aquaculture in Ecuador, as well as initial experiences of chame culture in Mexico, are conducted with wild caught juvenile fish. There is also interest in this fish in Nicaragua, where freshwater fishes such as tilapia are currently fetching higher prices than cultured shrimp. Therefore, the goals of this work are focused on the production of juveniles under laboratory conditions and minimize the dependency on wild fish supply. Available information indicates that in Ecuador, chame aquaculture has continuously decreased over the last eight years due to the shortage of juvenile fish since controlled propagation has not been achieved. Research in this area was largely abandoned over ten years ago, but most available references are available through a series of books, thesis and non-published reports from several institutions in Ecuador, with information on basic biology (Bonifaz *et al* 1985) and culture trials (Ecocostas, 2006) as well as reported production statistics of 1500 MT per year (FAO, 2009). For Mexico, there is a steadily demand on the central and the southern Pacific Coast. Also, as surveyed by the authors, there are already fish farmers interested in acquiring laboratory produced juveniles for commercial aquaculture in Oaxaca State, as a few preliminary growth-out trials have been conducted (Castro-Rivera *et al*, 2005). In addition, the species is not considered for protection under Mexican laws, and controlled juvenile production will provide a considerable benefit for the diversification of fish culture in Mexico. The main goals of this project are the following: 1) attempt hormonally induced reproduction by outlining the viability of the utilization of newer spawning techniques; 2) fertilization and egg incubation at different salinities to evaluate hatching success; 3) establish the viability of the initiation of exogenous feeding throughout a series of trials using both live and dry food as potential starter diets. This reports details the advances on the control of induced reproduction both in males and females, relevant details on the reproductive and early stages biology of the species and the results of the of larval rearing in laboratory conditions.

MATERIALS AND METHODS

Objective 1: Induced spawning and spermiation using synthetic analogues of luteinizing hormone releasing hormone (LHRHa).

Adult fish with a minimum size of 10 cm total length were collected within a 60 mile radius of Mazatlán Sinaloa, México and later transported and acclimated in FACIMAR-UAS facilities (23°12´57” N; 106°25´31” W). Fish were fed with a combination of 60% floating pellets (32% protein 8% lipids) and 40% sinking pellets (35% protein 10% lipid) (PURINA®). Fish of both genders were identified by the differences on the genital papilla and tagged using PIT-Tags (Passive Integrated Transponder tag, Biomark®) and potential breeders with visible signs of gonad maturation such as swollen abdomen, significant individual weight gain and changes in coloration on males and females, both in the papilla and the abdomen (Bonifaz *et al*, 1985; Estuardo

Campoverde, pers. comm.) were separated and monitored; however gonad biopsies were not possible due to the significantly reduced size of the pore at the papilla.

A series of experiments were conducted over two years. For year 1 (2010) 16 females were divided into the following groups: control group (0.5 ml/kg 0.9% saline solution), Desgly^{10}-Ala^{6} LHRHa injected at 40 µg/kg (priming dosage) and 80 µg/kg (resolving dose), or injections of Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®)., for year two (2011) 20 females divided into the following groups: control group (0.5 ml/kg 0.9% saline solution), Desgly^{10}-Ala^{6} LHRHa injected at 40 µg/kg (priming dosage) and 80 µg/kg (resolving dose), 2 injections of Ovaprim®, 0.5 ml/kg as priming dose and 1.0 ml/kg as resolving dose or a single implant 75 µg (Ovaplant®). In all cases, variables evaluated were spawning efficiency per group (%), oocyte size (um), and relative and total fecundity.
For males the conducted trials were as follows: for year 1, 16 males were divided into a control group (0.5 ml/kg 0.9% saline solution), LHRHa injected at 40 µg/kg, Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®); For year 2, 20 fish were used for an experiment was carried out using a control group (0.5 ml/kg 0.9% saline solution), either 40 or 80µg/kg Desgly^{10}-Ala^{6} LHRHa or Ovaprim® at 0.5 ml/kg at 5 fish per treatment. Studied variables for sperm quality were collected sperm volume, motility percent, and activation time and sperm concentration per treatment.

Objective 2. Effect of water salinity on fertilization, egg incubation and hatching success.
An protocol for sperm activation and fertilization as well as hatching success in terms of water salinity was conducted as follows: sperm samples were pre-diluted in ringer´s solution at several dilution ratios (1:1-1:40) (Arias-Rodriguez L. UJAT-Tabasco, per. comm.) as sperm viscosity was too high to allow effective activation with direct dilution in activation media (10 µm filtered, UV sterilized water). Once pre-dilution was completed, again 50-100 µl of Ringer´s diluted sperm samples were activated in 900 µl of activation media (0, 5, 15, 25, 35, 45, 55 and 65 ‰) to establish best activation conditions as water salinity value.

During year 1, once spawns were achieved, water salinity incubation conditions were estimated by placing 1000-1500 fertilized eggs in 1 l containers with 10 µm filtered, UV sterilized water at 0, 5, 15, 25, 35, 45, 55 and 65 ‰ with three replicas per salinity. Survival (%) per salinity and total length and morphological characteristics of larvae at hatching and thereafter were observed using digital image analysis with Motic Image Plus 2.0 software (Fig. 1).

For year 2, as continued study for optimal larval incubation and hatching conditions, 200 fertilized eggs were placed by triplicate in water previously adjusted to 0, 2, 4, 6, 8, 10 and 12 ‰ for later evaluation of hatching rate (%), total length yolk size diameter and oil drop size at hatching, again using digital image analysis with Motic Image Plus 2.0 software (Fig. 1), this procedure was repeated for several trials.

Objective 3: Effect of water salinity, food type and availability on larval survival and growth after yolk sack absorption.
For year 1, several trials were conducted using the following feeds: 50-80,000 cell/ml of *Chlorella* and *Scenedesmus* as freshwater phytoplankton, 50µm filtrated water (5-10 ml/day) from a rotifer *Brachionus rotundiformis* rearing tank, micropowdered *Spirulina* <20 µm (Mackay Marine®) (3 mg/l), Algamac 3000 1 m/lg , yeast 3 mg/l, microparticulate MPz < 70µm (Mackay Marine®) 1 mg/l, Epicin G2 (probiotic) as bacterial feed and biofilm promoter (3 mg/l), 60 l tanks with 15-20 ml/l of bioflocs, natural biofilm using shade cloth strips as substrate, and 35 µm filtrated Green-water originated in a 250 L tilapia bioflocs rearing tanks; for the trials, either a single or a combination of 2 or 3 treatments with four replicates per dietary treatment at a larval density of 0.25-0.5 larvae per ml, either on 1 l containers or at 50 l tanks. Alternatively, similar trials were

conducted considering an increment of 1.5 ‰ salinity per day using a combination of 2 or 3 of the previously mentioned feeds. Here, larvae from experiments were measured for survival, length increments, gut content (Rocha *et al.* 2008), and modified Fulton´s condition index (Sarnowski and Jezierska 2007), as well as fixation of larvae from several treatments in 10% buffered formalin for 24h and preserved in 75% ethanol afterwards. Several larvae were embedded in Historesin (Leica) and stained with Harris hematoxilin and eosin for optical analysis at the Aquaculture Center, UNESP Brazil.

For year 2, a new series of dry feeds as well as enriched rotifers were used in several feeding trials as follows: a first experiment using Artificial plankton 50 um particle size (Argent®) at 2 mg/l, ArteMac 0 (Aquafauna Bio-Marine Inc.®) at 4.5 mg/l, Encapsulon 0 rotifer substitute (30 um) 10 mg/l (Argent®) and Encapsulon 1 (50 um, Argent®) 20 mg/l; a second experiment using live rotifers as follows: control group (unenriched rotifers), enriched rotifers with Culture HUFA (Salt Creek Inc®), enriched rotifers with Protein HUFA (Salt Creek Inc®) and rotifers enriched with Algamac Protein Plus (Aquafauna Bio-Marine Inc.®) at a equal concentration of 5-8 rotifers per ml for all treatments, twice a day; both experiments were conducted at a rearing salinity of 0‰ in 1 l semi-clear plastic containers. Afterwards, three more experiments were conducted, with 2 trials using the same feeding treatments mentioned above (both live and dry feeds) but considering a gradual 1.5‰ salinity increment per replicate per day, starting with replicate 1 on the first day, both replicates 1 and 2 for the second day, etc. in 3 l clear plastic containers. At last, another experiment was conducted using a combination of 50,000 cels/ml of NANNO 3600 microalgae concentrate (Reed Mariculture Inc.) and 3 mg/l of Epicin (Epicore®) for all experimental units, using each one of the feeding treatments listed for the first experiment, with four replicates per feeding groups, each one at a different salinity of 2, 4, 6 and 8‰ in 1 l semi-clear plastic containers. For all cases, survival was estimated 6 days post hatching, and larvae from each feeding treatment and salinity were fixated either on 4% paraformaldehyde or Karnovski´s fixative for later histochemical analysis. Also, digital images of apparent digestive activity were obtained with a phase contrast optical microscope.

Objective 4. Studies on the utilization of artificial diets for weaning of chame larvae

Once larval feeding activity is observed, larvae will be fed with live rotifers *Brachionus plicatilis* and *Artemia* nauplii in a sequence as initial food and then 7 or 14 day old larvae/juveniles will be weaned to formulated diets as follows: (1) a commercial diet, (2) an experimental casein-gelatin based diet with maca meal as attractant, (3) an experimental diet based on freeze-dried preparation of fish muscle, (4) freshly hatched brine shrimp nauplii. Both experimental diets will be formulated based on our previous experience and will be isonitrogenous (protein requirement: 55% for most larval fish, Dabrowski 1986). At the end of the experiment, growth performance will be evaluated in terms of final individual body weight, survival (%), specific growth rate (SGR, %) and weight gain (%). Fish from each dietary treatment will also be sampled for proximate body analysis (water, protein, lipid, ash) if the size at the termination of the rearing period will permit (at least 0.5 g individual weight).

RESULTS

Objective 1.

For year 1, spawning results showed 80-100% success within 24h-48h for the Ovaplant group, 25-50% for LHRHa but 0% for Ovaprim within 48-72h. Obtained data describes as oocyte size as 300±50 µm and a relative fecundity of 80,000 to 100,000 cells per gram (Table 1). All LHRHa delivery treatments were effective to induce spermiation in volumes from 0.5 to 10 ml per male (LHRHa injected at 40 or 80 µg/kg, Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®); however several males released sperm naturally up to 1 ml throughout the reproductive season

(Table 3). Obtained data indicates that sperm activation time is close to 4 minutes, and overall concentration is within the range of 1 to $2X10^9$ cells per milliliter (Table 3). Sperm activation is highly improved after predilution on a 1:10-1:40 ratio in Ringer´s solution. No spermatocrit values were recorded as chame sperm viscosity probed to high in undiluted sperm.

For year 2, using one year captive broodstock, reproductive parameters did not change significantly from those observed on year 1. Again females from the Ovaplant group did spawn within 24-48 hours of implantation and females from the LHRHa treatment spawned 24-48 hours after injections, both at 80% efficiency (n=5 per treatment). Relative fecundity was close to 800,000 cells per female, with no natural spawns observed (Table 2). As sperm quality, there was an apparent reduction on released amounts per treatment, as well as reduction of 20% on activation values and 50-60% total activation time as well as estimated sperm concentration values in 30-40% (Table 4)

Objective 2.

As optimal salinity values, both for fertilization and egg incubation, our results indicate that there is no sperm activation above 5‰ of salinity. Data was recorded for optimal incubation salinity, as initially no hatching was observed above 15‰ salinity, although fertilized eggs can be transferred directly to salinities between 0-12‰ with no negative impact on hatching rate, total length (µm) yolk sack diameter and lipid drop among salinity treatments (Fig. 3). Therefore all fertilization procedures were conducted in 1 µm filtered, UV sterilized fresh water. The values of hatching size, yolk sack and oil drop diameter were not affected by rearing salinity on values of 0, 2, 4, 6, 8, 10 or 12 ‰ as observed in several trials (n=3). As example, for trial 1 values for total length at hatching were 1541.1±73.5 µm, yolk sack diameter 146.6±13.2 µm and oil drop 81.4±10.3 µm (Fig. 3). It is important to mention that larvae were photographed also at 3 and 5 days post hatching with interesting results as unfed larvae survival, given that most remaining larvae were found at salinities of 2, 4, 6 and 8 ‰.

As morphological description, eggs are transparent and spherical with a 300 µm average diameter and have an adhesive layer (Fig. 4a). Hatching occurs at 14-17 hours at 26°C, initial larvae length is close to 1288.2±137.2 µm, yolk sack diameter is around 171.2±10.6 µm with a single oil drop, no eyes or mouth are visible and show a vertical floating position with no active movement (Fig. 4b). At 24 h (1 day posthatching DPH), yolk sack diameter reduces to 137.1±8.3 µm, eyes are perceptible, with no pigmentation and digestive tract is noticeable (Fig. 4c). Mouth opening occurs at 2 DPH, ayes are well pigmented and digestive tract structures become more discernable (intestine, vestigial anus); yolk sack diameters is significantly smaller 93.8±10.7 µm (Fig. 4d). At 3 DPH, yolk sack is fully consumed and oral movements are perceptible and digestive tract has an evident circumvolution and pigmentation (Fig. 4e). Anus fully opens at 4 DPH, and some other internal structures are visible (i.e. liver) and body pigmentation increases considerably (Fig 4f).

Objective 3

Several feeding trials were conducted to attempt initiation of exogenous feeding. For year 1, larvae from all trials survived for only 5-7 days post fertilization at rearing temperature (26-29°C), with 100% mortality afterwards. Observed data both on length increment or condition index, were not significant different among diets or rearing salinity, figures 5a and 5b exemplify observed values for these two variables for larvae reared in a salinity gradient. For intestinal content, a few larvae showed particles inside the digestive track, particularly when feed in a combination of Algamac plus yeast or spirulina (Fig 6a). As mentioned a few larvae were processed for histological analysis (Fig. 9 and 10) with a few noticeable structures such as remnants of yolk inside the peritoneal cavity (Fig. 9) and the presence of an apparent digestive gland (pancreas) 6 days after hatching, although further analysis will be conducted for histochemistry on newly obtained larvae as continued studies for a preliminary depiction for enzymatic activity on early stages of chame larvae.

For year two, the inclusion of fresh water rotifers as live feed (non and bioenriched) as well as a new series of artificial diets, either in fresh water, salinity gradient of fixed salinities (2, 4, 6 and 8 ‰) did not showed different results in larvae survival, although as described in Figure 7, there was still a significant number of surviving larvae in two of four treatments, Artificial plankton and Artemac in combination with 50,000 cells/ml NANNNO 3600 and 3 mg/l of Epicin probiotic. At this moment the same trial is been replicated to substantiate this particular finding. Phase contrast microscopy analysis of live larvae fed with these two treatments allowed to observed large quantities of particles inside the digestive tract (Fig. 6b).

Objective 4

As no larvae has been successfully until the point of live feed consumption or beyond 7 days post hatching, no results were obtained for this particular objective; although at present moment feeding trails are still in progress.

DISCUSSION

The present report describes significant findings on the advancement of Chame broodstock and gamete management as well as egg incubation conditions as proposed in objectives 1 and 2. However, even that larvae survival has not been achieved beyond 8 days post hatching; there are important findings that will possibly allow to success in further studies.

Objective 1.

Induced spawning techniques are a reliable tool for laboratory condition reproduction of many fish species of relevance for aquaculture. As observed for both years, synthetic analogs of gonadotropin releasing factors such as GnRHa and LHRHa either by injection or implantation, did allow gamete release for both genders as successfully proven in many other species such as bullseye puffer both for males (Rodriguez M. de O., 2001) and females (Duncan *et al*, 2003), salmonids (Zohar and Mylonas, 2001), barramundi *Lates calcarifer* (García, 1989), stripped seabass *D. labrax* (Fornies *et al.,* 2001). Implants have an advantage as the continued and prolonged release of hormones is stipulated as 50% within 2-3 hours and the remaining hormone over 8-10 hours (Crim *et al*, 1988), being very advantageous on Chame induced reproduction. The product Ovaprim is a valid tool for induced reproduction in many fish species mostly catfish (Sahoo *et al*, 2007) and ornamental fish (Yanong *et al*, 2009); however for chame positive results were only observed for males as increased spermiation but no oocyte release in females; whereas dosages of 0.3-0.7 ml/kg had induce spawns in spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) (Kather Haniffa and Sridhar, 2002).

Purified gonadotropins such as hCG were not considered, as previous unplublished reports and personal communication form researchers from Ecuador mentioned dosages as high as 10,000 IU per fish, when in most cases required dosages are significantly lower (10 times) as reported for leopard grouper *Mycteroperca rosasea* (Gracia-Lopez *et al*, 2004) as well as potential immunoreactivity on treated fish, reducing or eliminating the effectiveness of the treatment (Patiño, 1997). Despite this information, other fish from the Eleotridae family do respond to spawning induction both with Ovaprim and HcG (Chorulon®) treatments as evaluated in *Gobiomorus dormitor* were 1500-3000 IU of HcG induced gamete release (Harris *et al*, 2011). Nevertheless we consider spawning induction with LHRHa either by injection or implantation more effective for Chame.

Reduced spermiation is a recurrent problem on males in captivity; nevertheless is not apparent in newly-capture Chame, although it increases when breeders, have been in captivity for over one year as observed in our experiment. The main difference was the reduction on several sperm quality

parameters such as released volume, motility, time of activity and concentration from year 1 to year 2; and yet sustaining the relevance of the use of LHRHa as a valid tool to induce sperm release in Chame with good quality as described for many other fish species (Zohar and Mylonas, 2001)

Objective 2.

Chame biology is quite interesting as salinity appears to play a major role on reproduction. Our findings indicate a low salinity tolerance both for sperm activation and fertilization, although both are closely related, hatching can occur at higher salinity values with no negative effect on larvae characteristics. Initially this factor was not considered relevant as larvae have been found in many environments; both brackish (Navarro-Rodriguez *et al*, 2006) and sea water (Franco-Gordo *et al*, 2002); thus reproductive biology of Chame occurs mostly in freshwater. It is not uncommon to find this type of reproductive performance in estuarine fishes, were fertilization occurs more efficiently at low salinities but hatching is not affected by the salinity gradient for egg incubation, as reported for *Fundulus heteroclitus* with better fertilization values around or below 15‰ and high hatching .percentage between 10-30‰ (Bush and Weis, 1983). Therefore the main mechanism for sperm activation in Chame is an osmotic change due to environmental conditions within 0-5‰, subsequently affecting fertilization.

Incubation salinity can significantly reduce larval survival and increase deformities as observed in *Anguilla japonica* (Okamoto *et al*, 2009). On a first trial (year 1) we observed zero % hatching at salinity values above 15‰; therefore for year 2 we conducted a second trial at reduced salinities, no negative effect was observed on hatching rates with values close to 95% in most cases, some fish despite of being present mostly in marine environments need a lower salinity value for early development such as *Takifugu obscures*, where preferred incubation salinities are between 0-8 ‰ (Yang and Cheng, 2006). Other fish, Australian bass *Macquaria novemaculeata*, have a similar behavior as Chame where adults are mostly present in freshwater; however, when eggs are incubated on salinities between 5-35‰ there in hatching on salinities below 25‰ (Van Der Wal, 1985).

There are a series of studies that mention the potential effects of rearing salinity on larvae size or yolk sack utilization. At selected salinities for year 2, Chame larvae did not show any differences on larval length, yolk sack and oil drop diameter, indicating no negative effect of such incubation salinities; on the other hand, the snapper *Pagrus auratus* (Steward Fielder *et al*, 2005) and yellowtail *Caranx mate* (Santerre 1973) exhibit reduced sizes and differences on yolk sack utilization in high salinities salinities (>35‰), although hatching was not affected by this factor. Todd (1975) described hatching lengths smaller than those observed in our study for Chame larvae as 0.8-0.9 mm, whereas Gaudé *et al* (2010) described hatching sizes of 1.13±0.01 mm for *Dormitator maculatus*; however both studies were conducted at temperatures of 23 or 13°C respectively, a 4-14°C difference with the present work. The last study also describes that yolk sack is not fully consumed until 9 dph at a rearing temperature of 13°C and 4.4‰ salinity. Chame larval length at hatching corresponds to values described for bigmouth sleeper *G. dormitor* at 1.0-1.5 mm but with significantly smaller yolk sack (Harris *et al*, 2011)

Objective 3.

Fish larvae first feeding after yolk sack depletion is essential for growth and survival. As observed in our study low survival rates are not uncommon at this particular stage (Dou *et al*, 2000), however zero survival observed in most conducted trials in this study by day 6-7 post hatching, can also be related to species resilience as reported for unfed sargus (*Diplodus sargus*) as larvae survived for up to 10 days (Darias *et al*, 2003), thus is quite possible that Chame larvae were not able to utilize offered feeds.

Observations of particle content in larvae digestive tracts, both for year 1 or 2 do not warranty digestion of such particles, probably due to poor enzymatic activity and a probable dependence of exogenous enzymes commonly present in live feeds (Cahu and Zambonino, 2001), however the lack of consumption of offered freshwater rotifers (year 2) and other zooplankton sources (year 1) do not provide enough evidence of the contribution of exogenous enzymes to ensure food utilization. Nevertheless, there is evidence of the feasibility of rearing larvae as small as Chame larvae, considering the evidence provided by Mata *et al*, (2004) where grunt Orthopristis ruber with an hatching size of 1.35±0.15 mm were successfully reared with a combination of *Isochrysis* (microalgae), *Brachionus plicatilis* and *Apocyclops distant* copepodids.

For year 1, we observed a perceptible preference for micropowdered spirulina particle ingestion, but still with no apparent utilization, while other fish larvae can be reared with this microalgae almost as successfully as when rotifers are used in knifefish *Chitala chitala* (Sarkar *et al*, 2006). Also, both for year 1 and 2, the use of probiotics or yeast did have as purpose to estimulate digestive activity, growth and metabolism as food utilization (Vine, 2006; Getasuope, 2007), but with no relevant results for year 1. On the other hand, as promising results the combination of microalgae concentrate, probiotic and artificial feeds, with some significant survival up to 6 dph during the last conducted experiment provides some tentative course to continue other similar feeding trials for the remaining of 2011 Chame reproductive season. Addition of microalgae can potentially improve larvae survival by providing nutrients (Naas *et al*, 1996) or visual contrast, attractants and probiotics (Olsen *et al,* 2000).

Conclusion

More research is needed to achieve Chame larval rearing, as at this moment particle ingestion of several kinds of artificial feeds has been assured after macroscopical analysis, both on fixated and live larvae. Also more research is required into obtaining a suitable live feed prey or preys along with the simultaneous evaluation of a wider selection of artificial feeds.

ACKNOWLEDGEMENTS

We are indebted to Joselito Audevez and Omar Serna for technical assistance at FACIMAR Mazatlán México and Prof. Fernando Zara, of the Invertebrate Morphology laboratory at UNESP Jaboticabal Brazil for facilitating the equipment for larvae histology.

REFERENCES

Bonifaz, N; Campos, M; Castelo, R; 1985. El Chame, una nueva fuente de alimentación e ingresos. Fundación Ciencia. 173 pp.

Bush, C.P. Weis, J.S. 1983. Effect of salinity on fertilization success in two populations of F*undulus heteroclitus*. Biol. Bull. 164:406-417.

Cahu, C., Zambonino, J. 2001. Substitution of live food by formulated diets in marine fish larvae. *Aquaculture,* 200: 161-180.

Castro-Rivera, R., Aguilar-Benítez, G.,. Hernández- Girón, J. P. 2005. Conversión alimenticia en engordas puras y mixtas de Popoyote (*Dormitator latifrons* Richardson) en estanques de cemento. Revista AquaTIC 23:45-52.

Crim LW, Sherwood NM, Wilson CE. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 74: 87-95.

Darias, M., Pacual, E., Cárdenas, S., y Yúfera, M. 2003. Metabolismo energético de huevos y larvas sin alimentar del sargo (*Diplodus sargus*) en cultivo. IX Congreso Nacional de Acuacultura. Cadiz 2003.

Dou, S.Z., Seikai, T. y Tsukamoto, K., 2000. Cannibalism in Japanese flounder juveniles, *Paralichthys olivaceus*, reared under controlled conditions. Aquaculture 182:149–159.

Duncan, N.J, G. A. Rodriguez M de O, D. Alok, Y. Zohar. 2003. Effects of controlled delivery and acute injections of LHRHa on bullseye puffer fish (*Sphoeroides annulatus*) spawning. Aquaculture 218, 625-635.

EcoCostas. 2006. El Cultivo de Chame (*Dormitator latifrons*) en el Estuario del Río Cojimíes. Proyecto para la Conservación y Desarrollo del Estuario de Cojimíes (Sustainable Coastal Communities and Ecosystem Project -SUCCESS). Pp. 24.

FAO, Fishstat Plus. 2009. Aquaculture production 1950-2009. [18 de Abril 2011]. URL:http://www.fao.org/fishery/statistics/software/fishstat

Fornies MA, Mananos E, Carrillo M, Rocha A, Laureau S, Mylonas CC, Zohar Y, Zanuy S. 2001. Spawning induction of individual European sea bass females (*Dicentrarchus labrax*) using different GnRHa delivery systems. Aquaculture 202: 221-234.

Franco-Gordo, C., Godinez-Dominguez, E., Suarez-Morales, E. 2002. Larval fish assemblages in water off the central Pacific coast of Mexico. Journal of Plankton Research 24:775-784.

Gatasoupe, F.J. 2007. Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture, 267: 20-30.

Gaudé, A., Brown, C., Green, C. 2010. Awakening the sleeper: bringing *Dormitator maculatus* into full market potential as a premier marine bait for Louisiana. Abstract Aquaculture 2010. San Diego Californa, USA.

Gracia-Lopez, V., Rodriguez-Romero, J., Perez-Ramirez, J. 2004. Inducción del desove con HCG y desarrollo embrionario y de larvas de la cabrilla sardinera *Mycteroperca rosasea* (Streets, 1877). Ciencias Marinas, 30:279-284.

Harris, N., Wesley Neal, J., Perschbacher, P.W., Mace C.E., Muñoz-Hincapíe, M. 2011. Notes on hatchery spawning methods for bigmouth sleeper *Gobiomorus dormitor*. Aquaculture Research. 42:1145-1152.

Kather Haniffa, M.A., Sridhar, S. 2002. Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadotropin and synthetic hormone (ovaprim). Veterinarski Arch 72:51-56.

Mata, E., Rosas, J., Velasquez, A., Cabrera, T. 2004. Hormonal induction to spawning and larval descritption of the grunt *Orthopristis ruber* Cuvier (Pisces: Haemulidae) . Revista de Biología Marina y Oceanografía 39:21-29.

Naas, K., Huse, I. y Iglesias, J. 1996. Illumination in first feeding tanks for marine fish larvae. Aquacultural Engineering, 15:291-300.

Navarro-Rodríguez, M.C., González-Guevara, L. F., Flores-Vargas, R., González-Ruelas, M. E. Carrillo-González, F. M. 2006. Composición y variabilidad del ictioplancton de la laguna El Quelele, Nayarit, México Ichthyoplankton composition and variability of the laguna El Quelele, Nayarit, Mexico. Revista de Biología Marina y Oceanografía 41: 35 – 43.

Okamoto T, Kurokawa T, Gen K, Murashita K, Nomura K, Kim SH, Matsubara H, Ohta H, Tanaka H (2009), Influence of salinity on morphological deformities in cultured larvae of Japanese eel, *Anguilla japonica*, at completion of yolk resorption. Aquaculture, 293: 113-118.

Olsen, A.I., Olsen, Y., Attramadal, Y., Christie, K., Birkbeck, T.H., Skjermo, J. y Vadstein, O. 2000. Effect of short term feeding of microalgae on the bacterial flora associated with juvenile *Artemia franciscana*. Aquaculture. 190: 11-25.

Patino, R., 1997. Manipulations of the reproductive system of fishes by means of exogenous chemicals. The Progressive Fish-Culturist. 59:18-128.

Rocha, R.J., Ribeiro L., Costa R., Dinis M.T. 2008. Does the presence of microalgae influence fish larvae prey capture? Aquaculture Research 39: 362-369

Rodriguez M de O, G.A. 2001. Evaluación de la calidad del esperma del botete Diana *Sphoeroides annulatus* (Jenyns, 1834) en condiciones de cautiverio y bajo inducción hormonal con LHRHa. Masters degree Thesis. CIAD, A.C. Mazatlán Sin.

Sahoo, S.K., Giri, S.S., chandra, S., Sahu, A.K. 2007. Effect of Ovaprim doses and latency periods on induced spawning of *Clarias batrachus*: observation on larval deformity. Indian Journal of Experimental Biology, 45:920-922.

Santerre M.T. 1976. Effects of temperature and salinity on the eggs and early larvae of *Caranx mate* (Cuv. & Valenc.) (pisces: carangidae) in Hawaii Journal of Experimental Marine Biology and Ecology, 21:51-68.

Sarkar, U.K., Lakra, W.S., Deepak, P.K., Negi, R.S., Paul, S.K. y Srivastava, A. 2006. Performance of different types of diets on experimental larval rearing of endangered *Chitala chitala* (Hamilton) in recirculatory system. Aquaculture, 261: 141-150.

Sarnowski, P., Jezierska, B. 2007. A new coefficient for evaluation of condition of fish larvae. Electronic Journal of Ichthyology. 2:69-76.

Steward Fielder D., Bardsley William J., Geoff L. Allan, Patricia M. (2005). The effects of salinity and temperature on growth and survival of Australian snapper, *Pagrus auratus* larvae. Aquaculture, 250: 201-214.

Todd, E.S. 1975. Vertical movement and development of the prolarvae of the eleotrid fish, *Dormitator latifrons*. Copeia 3: 564-568.

Van Der Wal, T. 1985. Effects of temperature and salinity on the hatch rate and survival of australian bass (*Macquaria novemaculeata*) eggs and yolk-sac larvae. Aquaculture, 47: 239-244.

Vine, N.G., Leukes, W.D. y Kaiser, H. 2006. Probiotics in marine larviculture. FEMS *Microbiol Rev*. 30: 404–27.

Yang Z, Y. Chen. 2006. Salinity tolerance of embryos of obscure puffer *Takifugu obscures*. Aquaculture, 253: 393-397.

Yanong, R.P.E, Martinez, C., Watson, C.A. 2009. Use of Ovarpim in Ornamental Fish Aquuaculture. University of Florida IFAS Extension. FA161, 8 pp.

Zohar, Y. y C. Mylonas. 2001. Endocrine manipulations of spawning in culture fish: from hormones to genes. Aquaculture, 197: 99-136.

Figure 1. Chame *Dormitator latifrons* (Photo by Gustavo Rodriguez)

Figure 2. New hatched larvae scanned with digital analysis tools (Motic Image Plus 2.0 software) (note: Scale bars not accurate) (Photo by Eva Medina)

Table 1. Estimated values of spawning females for all experimental treatments within the experiment for year 1 (2010).

Variable	Control	LHRHa	Ovaprim	Ovaplant
Weight (g)	393.3±185.1	486.15±205.2	388.9±151.6	388.6±216.1
#of fish per treatment	4	4	4	4
% of spawning fish	25%	50%	0%	100%*
Relative fecundity (cell g^{-1})	83000[c]	59000[c]	n/a	50000±10000**
Oocyte diameter (µm)	392.6±51.8[c]	327.7±18.5[c]	n/a	353.8±106.6**

*n=4 **Pooled from 4 females
[c]n=1 female

Table 2. Estimated values of spawning females for all experimental treatments within the experiment for year 2 (2011).

Variable	Control	Ovaprim	LHRHa	Ovaplant
Weight (g)	438.6±153.58	467±242.49	526.8±171.32	539.4±229.73
# of fish per treatment	5	5	5	5
% spawning fish	0%	0%	80%	80%
Relative fecundity (cell g^{-1})	n/a	n/a	50598.3± 5134.35	54310.3± 1639.4
Total fecundity per fish	n/a	n/a	691841± 637549	428541 ± 275455
Oocyte diameter (μm)	n/a	n/a	273.33± 0.10	273.55±1.51

Table 3. Estimated values of sperm quality for all experimental treatments for year 1 (2010)

Variable	Control	LHRHa (40μg kg^{-1})	Ovaprim	Ovaplant
Weight (g)	622.7±54.7	434.7±139.4	538.75±187.4	540.6±202.1
# of spermiating fish	3	4	3	4
Mean volume ml	0.5	2.3	4.3	8.2
Motility (%)	93.3±11.5	83.3±11.5	80.0±26.4	93.3±5.77
Activation time (min)	4:24±0:22	4:57±1:91	2:47±1:37	2:90±1:02
Concentration(cell ml^{-1})	1.96E+09± 1.29E+09	2.29E+09± 7.8E+08	1.26E+09± 2.37E+08	2.31E+09± 7.84E+08

Table 4. Estimated values of sperm quality for all experimental treatments for year 2 (2011)

Variable	Control	Ovaprim	LHRHa (40μg kg^{-1})	LHRHa (80μg kg^{-1})
Weight (g)	560.2 ± 65.14	554.6 ± 122.65	550.8±205.06	737.4±204.4
# Spermiating fish	5	5	5	5
Released volume (ml)	0.25±0.2	0.28±0.3	0.77±0.6	4.24±7.4
Motility %	60 ± 14.14	66 ± 30	76 ± 24	62 ± 0.27
Activation time (min)	1:63± 0:41	02:16± 1:15	1:47 ± 0.41	0:83±0:45
Concentration (cell ml^{-1})	1.04E+09± 6.93E+08	1.11E+09± 3.90E+08	1.52E+09± 8.25E+08	1.65E+09± 4.65E+08

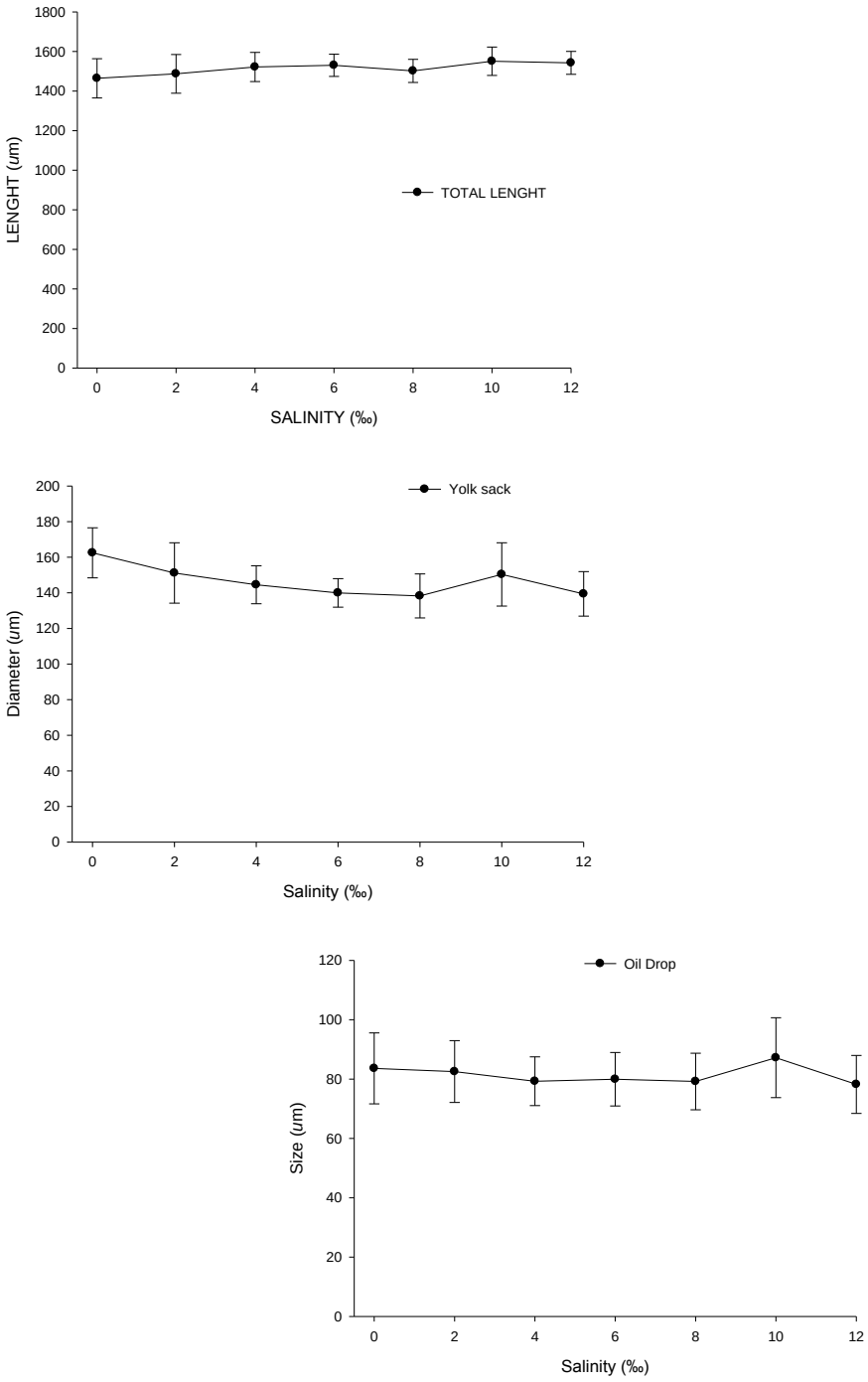


Figure 3. Observed values for hatching length (a), yolk sac (b) and oil drop (c) diameter in chame larvae incubated in salinities between 0-12 ‰, 12-16 hours post hatching.

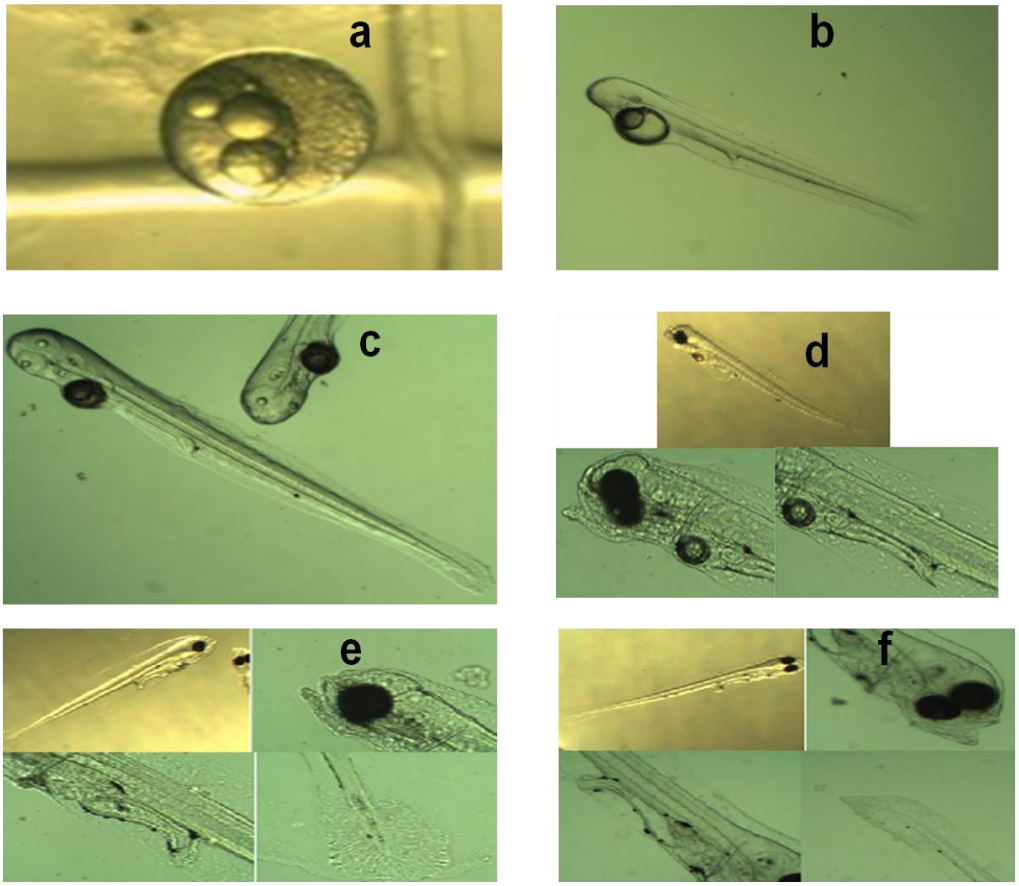


Figure 4. Early morphological development of chame larvae at 26-28°C. (40-400x)

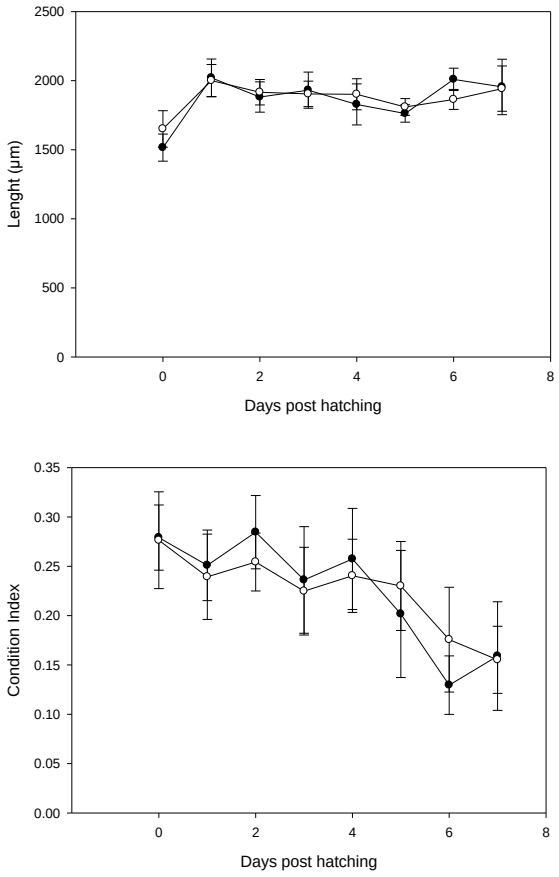


Figure 5. Length increment (µm) and progression of condition index (CI) of larvae reared at 1.5 ‰ increments from 1dph (○) or from 2 dph (●).

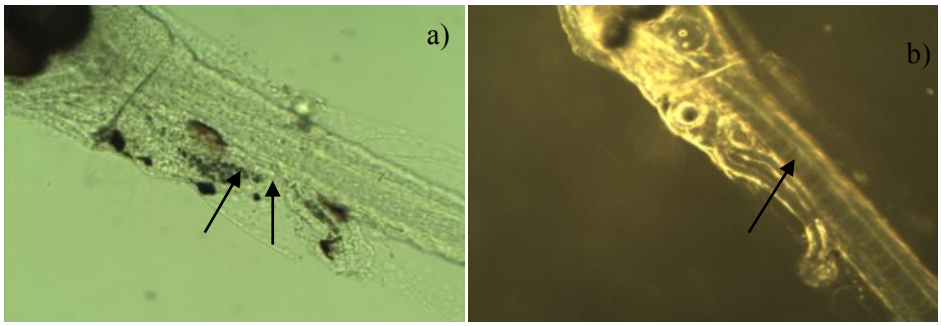


Figure 6. Observed digestive tract content (→) observed after staining Year 1 (a) or phase contrast microscopy Year 2 (400x).

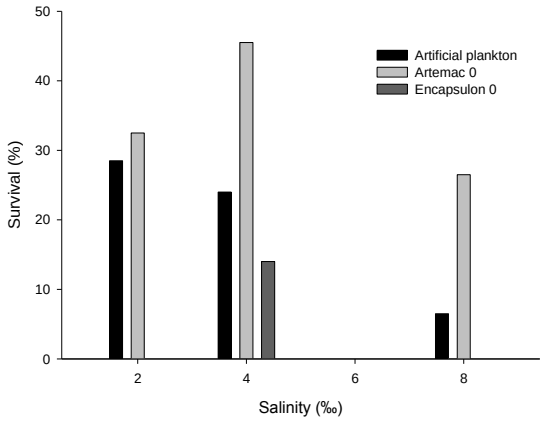


Figure 7. Chame larvae survival (%) observed during last feeding experiment at day 6 after initiation at each rearing salinity per treatment, n=200 larvae per container

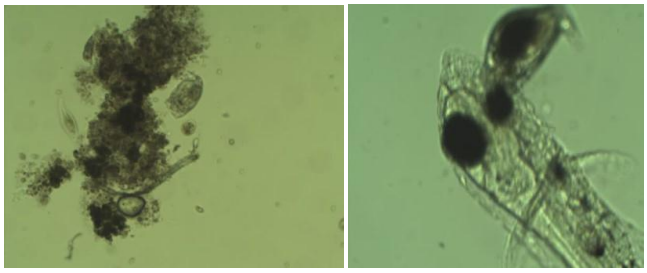


Figure 8. Biofloc produced for Chame larvae rearing and predation by present rotifers on bioflocs.

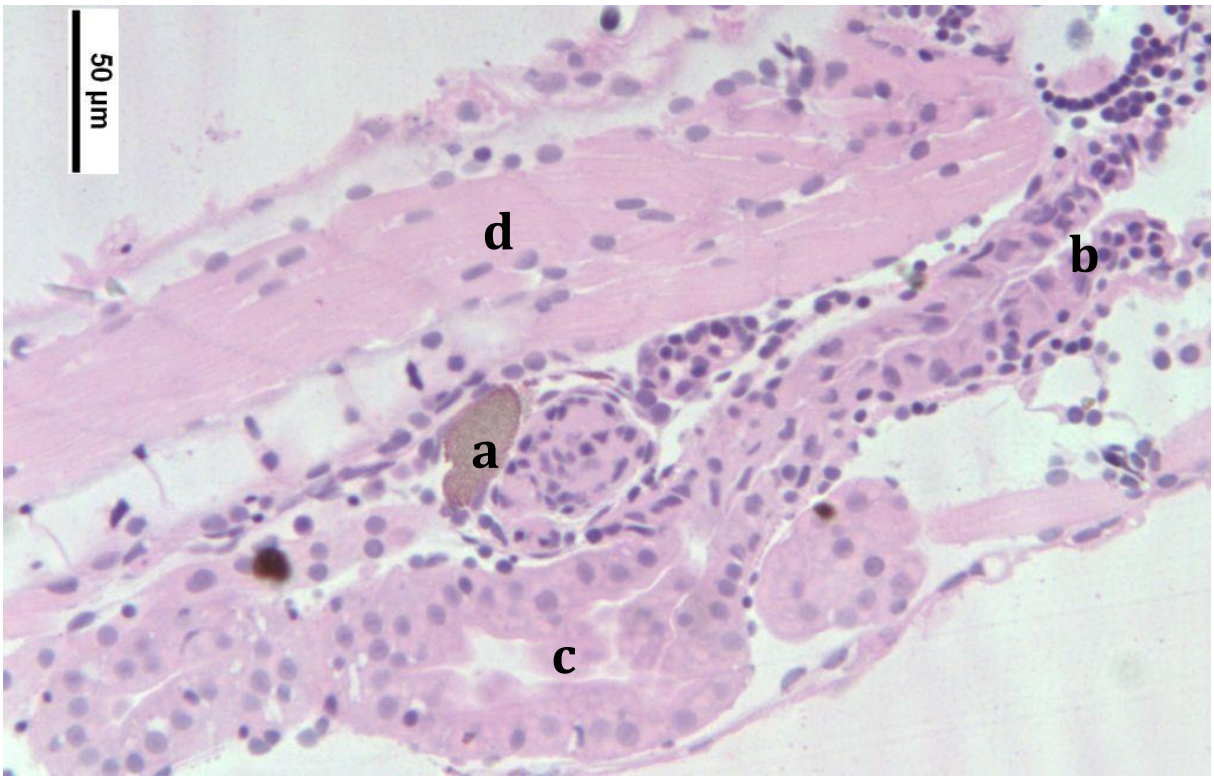


Figure 9. Chame larvae samples embedded in historesin and stained with Harris hematoxilin and eosin, observed structures are yolk sack residues (a), esophagus (b), stomach (c), muscle fibers (d) (400x).

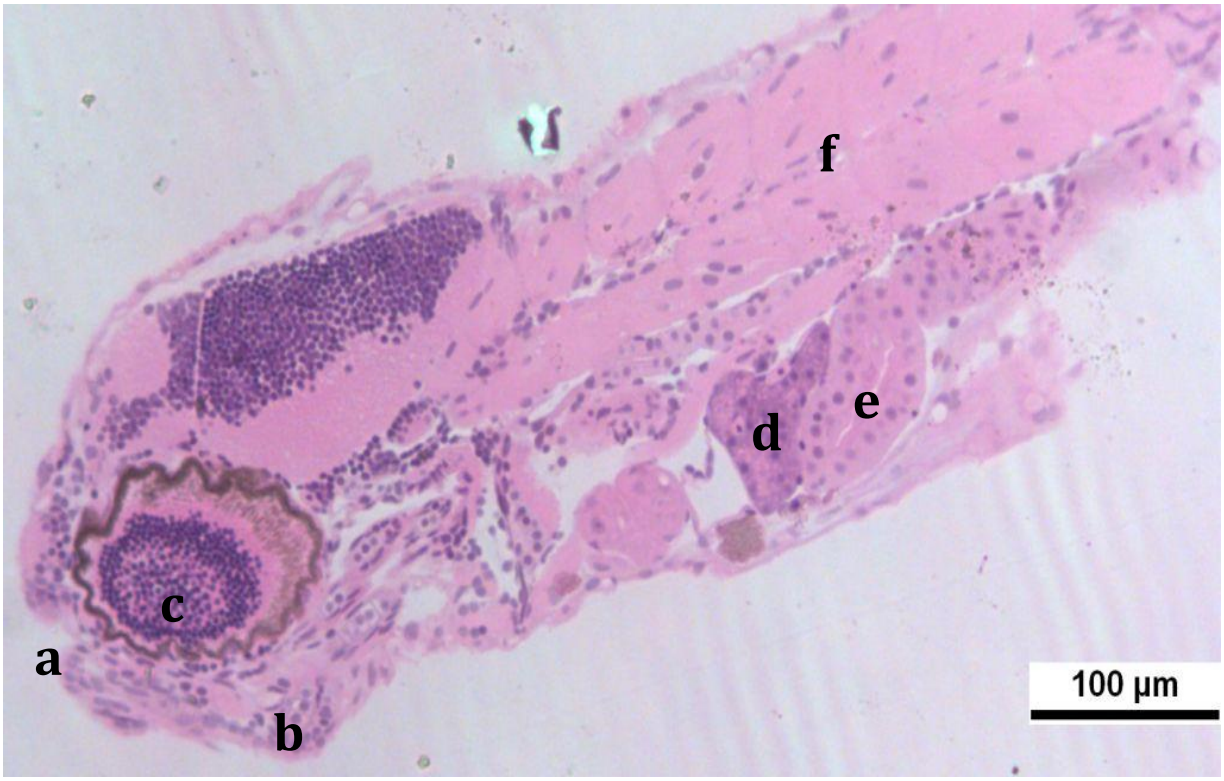


Figure 10. Chame larvae samples embedded in historesin and stained with Harris hematoxilin and eosin, observed structures are mouth (a), jaw (b) eye (c), pancreas (d), stomach (e), muscle fibers (f) (400x).

Stock Assessment of "Chame" *Dormitator latifrons* in Nayarit and South of Sinaloa México

Indigenous Species Development /Study/09IND04UH

Guillermo Rodriguez Domínguez
Universidad Autónoma de Sinaloa – Mazatlán
Mazatlán, Sinaloa, Mexico

Maria Haws
University of Hawai'i at Hilo
Hilo, Hawai'i, USA

Students:
Jesus Sanchez Palacios, Gloria Ana María Arroyo Bustos and Josefina Ruiz Moreno
Universidad Autónoma de Sinaloa – Mazatlán
Mazatlán, Sinaloa, Mexico

INTRODUCTION

"Chame" is a common euryhaline fish of Mexico, and much of the Pacific Coast of Latin America. It survives in a large range of temperatures and salinities in lentic and lotic environments. It is used for food in southern Mexico, but is considered a pest in northwest Mexico because it invades commercial shrimp ponds where it competes for feed. This fish has a vascularised swim bladder that can function as a primitive lung (facultative lung) and thus can survive a long time out of water. Because of its resiliency to extreme environmental conditions it will be important species if global warming is a reality. Its cultivation for human food may be an alternative when other species are impacted by global climate change.

Relatively little is known about this species' biology. A reproductive autumn season was reported (Navarro Rodríguez et al, 2004; Navarro Rodríguez et al, 2010; Rojas Herrera et al. 2009) but a reproductive winter season was also reported (Florencio y Serrano, 1981). Another study found two reproductive seasons a year (Navarro Rodríguez et al., 2006). Isometric growth (Rojas Herrera et al. 2009) and growth of 24 g in two and half months of cultivation was reported (Castro Rivera et al. 2005). A female of 155 mm length can produce five millions eggs (Haz Alvarado 2002). It spawns in estuaries where mass eggs adhere to the roots of the mangroves and then the 20 mm larvae migrate upstream (Haz Alvarado, 2002).

The principle objective of this study is to collect information about this species' basic biology and conduct a stock assessment in two Mexican States where this species is fished and has potential as an aquaculture species. This information will support development of fisheries management recommendations and provide information to aquaculture development efforts.

MATERIAL AND METHODS

Survey catches of "chame" were conducted using cast nets with 1 inch mesh size in rivers, streams, coastal lagoons estuaries and marshes of southern Sinaloa and northern Nayarit Mexico (Figure 1). In each survey site 10 catch sets were realized and the number of fish caught recorded. Sex, total length, total weight, and gonad weight were also recorded. Scales and gonads were collected from five fishes from each 10 mm size class.

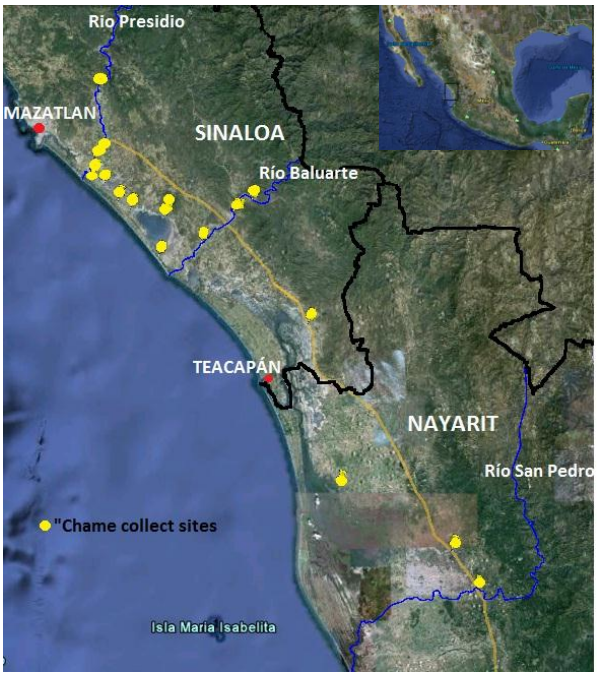


Figure 1. Study area and collection sites of "Chame" in southern Sinaloa and northern Nayarit.

Cleaned scales were air-dried, mounted on microscopic slides and examined with a Bausch and Lomb overhead projector. Six of the best scales were selected for further analysis. For each fish scale, total and partial radius were measured using a projection scale at a constant distance and microscopic objective for two independent observers To determine if scales could be used for estimation of growth, the total radius of the scale was regressed to the total length of fish and statistical significance was determined using analysis of variance. Lengths and weights of fishes were grouped for the number of growth marks and fitted to a normal distribution, and the parameters μ and σ were estimated for each one. The periodicity of growth marks was evaluated by examining mean marginal increments of the scales. To validate the timing of mark formation, monthly mean Fulton's condition factor ($\overline{K}$) and the gonadosomatic index were estimated.

Data for size at age were fitted to four cases of Schnute's (1981) model and one special case equivalent to the von Bertalanfy model. Each case was fitted to the size at age and weight at age data set by the maximum log-likelihood algorithm of normal distribution of errors, assuming both additive and multiplicative error structure. The best fit of error structure and of each case for the Schnute model was determined by the Akaike index (*AIC*) and the Akaike weights (W_i) (Montgomery et el 2010).

The relationship between weight (Wt) and length (Lt) of fishes was estimated by a potential model. The model was developed using non-lineal methods maximumizing a Log-likelihood function by a iterative process with Newton algorithm. Models were bootstrapped 1000 times to estimate first-order corrected 95% confidence intervals about each parameter (Haddon 2001).

Maturation stage were determined macroscopically in the laboratory using freshly captured specimens,. Samples of gonads were also taken and placed in Davidson´s fluid for subsequent histological analysis. Macroscopic maturation stages were: Stage 1. immature and sex

undifferentiated; Stage 2. gonads developing; Stage 3. gonads maturating; Stage 4. mature; and Stage 5. spawing. Stages 3 to 5 were considerate to be mature fishes during reproduction season. Fishes collected in the reproduction season were sorted as immature and mature for each 5 mm of total length interval and adjusted to the logistic model to estimate size at first maturity.

Fish caught at all survey sites and for all months were standardized as catch per 10 sets and pooled in 10 mm of total length interval. Then frequency in each age group (N*a*) was calculated by a multinomial approximation (Montgomery et al., 2010) where initial parameter estimates were that of normal distribution fit at each age group as determined by the growth marks in scales. Multinomial fit was obtained by fixing mean length at each age group by the change in standard deviation and frequency (N*a*). Then *Na* was used to construct a plot of catch at age curve, and the points in the descendent part of curve were selected for regression and estimation of total mortality rate, as $Ln(Na) = a + Z\bar{t}$

Mortality rate (M) was calculated with four empiric model as:

Reference	Model	Parameters definition and units
Rickhter and Efanov (1976)	$M = \frac{1.521}{t_{50\%}{}^{0.72}} - 0.155$	$t_{50\%}$ = First age maturity.
Rickhter and Efanov (1976)	$Log_{10}M$=-0.0066-0.27$Log_{10}L\infty$+0.6543$Log_{10}K$ +0.4634$Log_{10}T$	L∞ = asymptotic length (in cm) K = growth coefficient (year^{-1}) T = annual mean temperature of habitat. (◦C)
Hewitt and Hoenig (2005)	1.5 ($3/a_{max}$)	a_{max} = Maximum age registered.
Cubillos (2003) after Alverson & Carney (1975)	$M = \frac{3K(1-w)}{w}$	w = 0.62 = critic size-asymptotic length ration. K = (growth coefficient (year^{-1}) from vonBertalanfy model.

RESULTS

Chame were caught in ten of the eighteen sites explored. *D. latifrons* was collected all year in freshwater ponds or seasonal freshwater lagoons, and in rivers, streams and estuaries during rainy season from August to November. The catch per set was highly variable depending on the local situation and seasonal conditions. In a seasonal freshwater called Mataderos, catch per set (19.63 m^2 cast net area) was 5 to 10 fishes from March to May (Figure 2). The lagoon dried up in June, but it was filled in a flood in August, and after new chame recruits entered the lagoon, catches per set were 2.6 to 8.5 fishes. In a pond constructed for a source of water for cattle (named Zacatoza), which had been filled continuously with well water, catch per set was 0.05 to 1.8 fishes from March to August. In late August the surrounding fields were flooded and the reservoir turned dark brown and dead fish were observed on the surface. In October, new recruits of chame were observed, and catch per set was from 1.8 to 44.5 fish, from October to December. In a stream next to Potrerillos ranch, many chame were observing attempting to migrate upstream but a bridge over the stream presented this. Some fishes were seen attempting to jump over the wall but were returning with water flow. Catch per set was of 28.3 fishes. In early December, the flow of water was reduced and there only remained a small lagoon next to the bridge where catch per set was of 128 fishes.

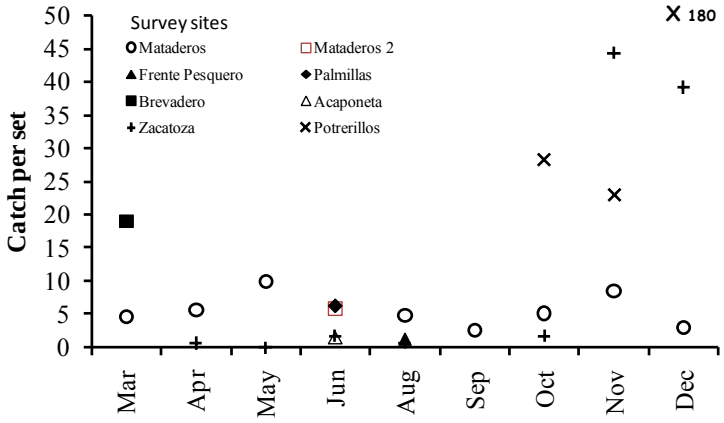


Figure 2. Catch per set of chame (*D. latifrons*) with a cast net in survey sites in southern Sinaloa and northern Nayarit, México.

The observed migration is thought to have been for reproduction because in October 75% of female fishes were mature (stage 4) and for November 87.5% had spawned. In September, chame were captured next to the dam diversion "Tamarindo" on the Baluarte River; 67% were mature. Some smaller fish were observed hiding beneath rocks at this site. Catch per set was not calculated because the rocks prevented the use of cast nets, but 86 chame fishes were captured in 30 minutes for two fishers.

The size of chame captured varied from 56 to 300 mm of total length, but most (79%) were in the 70 to 130 mm interval (Figure 3). A decrease of frequency with size was observed. The weight of the chame varied from 1.5 g to 419 g but most (76.8%) were 40 g (Figure 3).

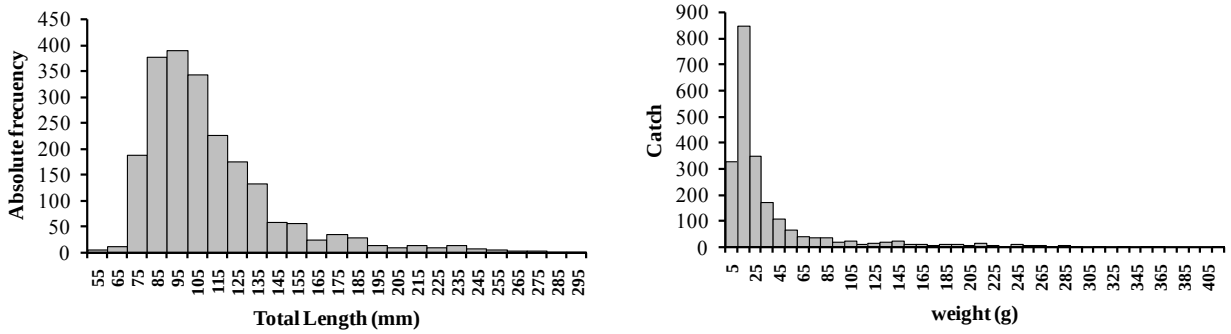


Figure 3. Size and weight structure of *D. latifrons* as sum of standardized catch to 10 set of all survey sites.

The relationship between total length and scale size was statistically significant ($p < 0.05$) with a coefficient of determination of 0.92. This lends validity to the use of scales for age determination. Six growth marks were found on the scales (Figure 4) and the total length of fishes within each growth mark was adjusted to a normal distribution.

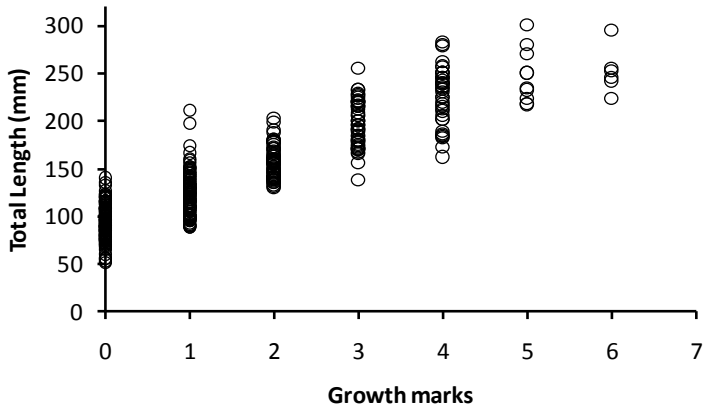


Figure 4. Relation between total length and growth marks in scales of *D. latifrons*.

The mean of the marginal increments described an annual cycle with the lowest means in December and February. (Figure 5). This find validates the hypothesis that growth marks are imprinted once a year from December to February.

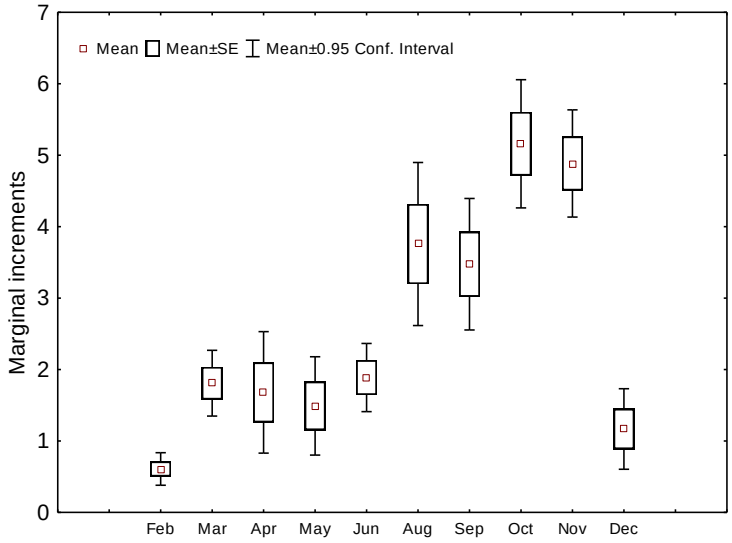


Figure 5. Temporal variation of mean marginal increments of scales of *D. latifrons*. Fishes of all groups of growth marks were included.

Total length and weight for the age groups were as:

age group	Mean total length (CI 95%)	weigth (CI 95%)
0	91(88,94)	13(15,17)
1	120(116,124)	29(33,38)
2	155(151,159)	53(59,65)
3	195(189,202)	123(136,150)
4	227(217,236)	187(213,240)
5	246(230,263)	228(285,341)
6	254(237,272)	187(279,371)

The best fit of growth of size at age was case 1 with additive error structure as was determined for the lower AIK (Table 1).

Table 1. Akaike index after fitting for Schnute model cases and error structure types to size-age data.

ERROR TYPE	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
ADITIVE	34.50	55.39	61.37	66.32	59.17
MULTIPLICATIVE	59.36	62.01	66.37	70.21	64.77

Aikake weight for case 1 has additive error structure being almost 100% as is shown in Tables 2 and 3.

Table 2. Parameters of each case of the Schnute model with bootstrapped confident interval 95% for the best model as determined by Aikake weight.

CASE	y1	y2	a	b	AIK	W_i
1	91.2 (90.48, 91.58)	254.38 (253.71, 254.79)	1.15 (1.08, 1.2)	-4.08 (-4.46, -3.86)	34.50	99.99 *
2	86.31	259.81	0.34	0.00	55.39	0.0
3	87.01	264.17	0.00	1.68	61.37	0.0
4	113.13	276.45	0.00	0.00	66.32	0.0
5	85.83	261.58	0.14	1.00	59.17	0.0

Table 3. Akaike index after fitting five cases of the Schnute model and two types of error structure to weght-age data.

ERROR TYPE	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
ADITIVE	74.05	70.43	81.83	80.48	84.37
MULTIPLICATI	74.10	78.90	82.46	85.70	84.26

The best fit of growth to weight at age was case 2 with additive error structure as was determined for the lower AIC (Table 3) and Aikake weight was 85.16% (Table 4).

The length-weight relation was: $\mathrm{Wt} = 0.0000143\mathrm{Lt}^{3.019}$. Bootstrapped confident intervals at 95% were 1.18E-5 – 1.62E-5 for parameter *a* and 2.984 – 3.051 for parameter *b*. Confident interval at 95% of *b* include 3 which indicates isometric growth of *D. latifrons*.

Table 4. Parameters of each case of the Schnute model with bootstrapped confident interval 95% for the best model as determined by Aikake weight

CASE	y1	y2	a	b	AIK	W_i
1	15.86	285.34	2.22	-3.15	74.05	13.92
2	1.07 (1, 5.63)	294.45 (279.43, 314.05)	0.64 (0.4, 0.68)	0.00	70.43	85.16 *
3	2.00	294.45	0.00	1.18	81.83	0.28
5	1.97	371.64	-0.19	1.00	84.37	0.08

The condition factor of chame fishes showed an annual cycle according to gonadosomatic index and marginal increments in scales. The lowest values were in March with a maximumum in October, followed by a decreasing trend until December (Figure 6).

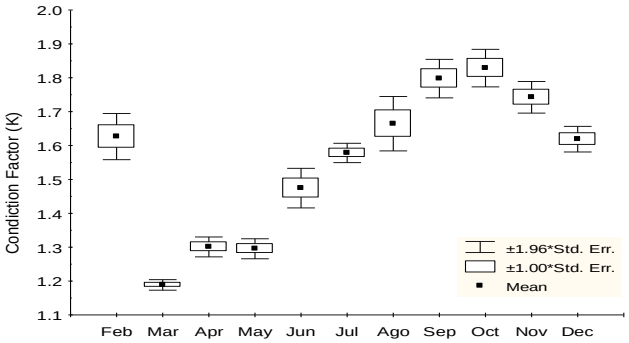


Figure 6. Temporal variation of condition factor for Chame *D. latifrons*.

Macroscopic analysis of the maturity stage of female Chame showed an annual maturation cycle. From March to June, females were inmature or developing. From August to November, most fish were mature. Stage 5 or the spawned stage were seen from November to February (Figure 7).

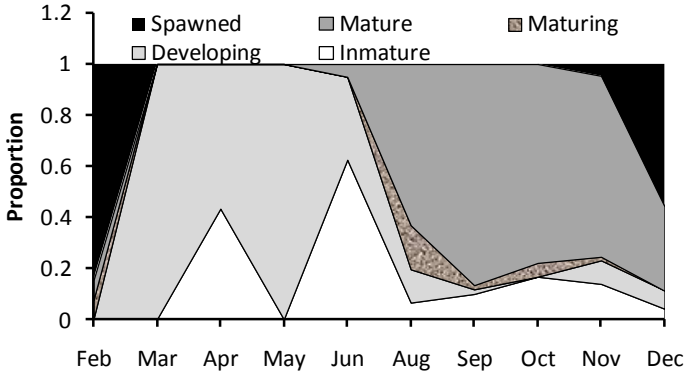


Figure 7. Proportional macroscopic maturing stages of *D. latifrons* over the annual cycle.

Temporal variation of mean GI also revealed an annual maturity cycle (Figure 8). From February to July, GI was low, ranging between 0.5 and 2. Beginning in August, GI began to increase (from 4 to 14) until reaching a maximumum in October, followed by a decline until December. Both the macroscopic maturity stages and GI suggest that the spawning season for chame is from October to February.

Female chame with zero growth marks also showed gonadic maturation as revealed by the gonadosomatic index. This find revealed that *D. latifrons* reach first maturity before they reach one year of age, because the growth mark is impressed in December and these fishes would have been maturating since August. There was only one female with zero growth marks in December that was in spawned condition and one more was found in November. A weighted mean age (frequency times age) revealed that females with zero growth marks collected during this study were 0.845

years old. Mean size of mature females with zero growth marks was 98 mm total length, and those that were immature were 86 mm.

Eggs in gonads of *D. latifrons* are homogeneous in size when are seen in fresh under the microscope and histological analysis of gonads revealed eggs in the same development stage (Figure 9), which validates the hypothesis that *D. latifrons* is a complete spawner.

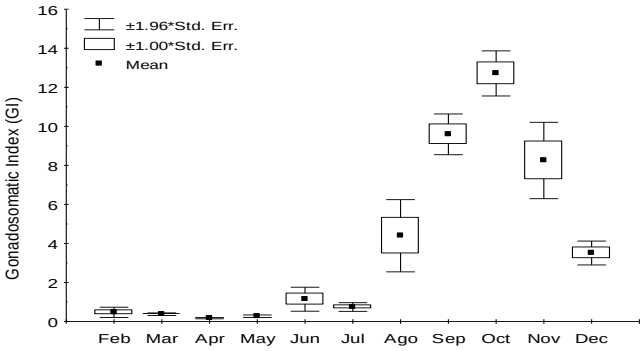


Figure 8. Annual cycle for gonadosomatic index of *D. latifrons*.

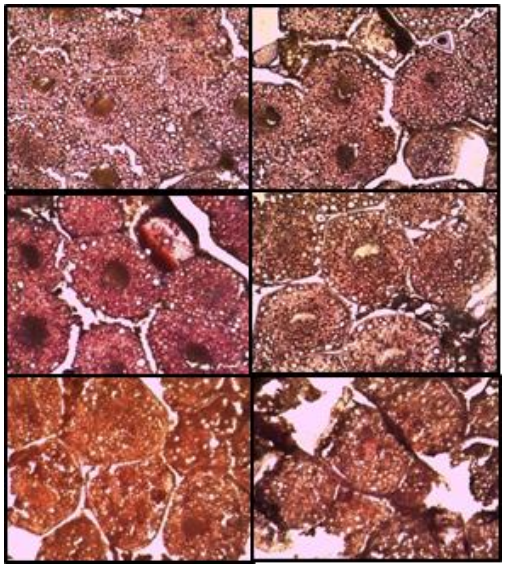


Figure 9. Histological view of mature gonads of *D. latifrons* female of 119 mm (top), female of 115 mm (middle) and female of 90 mm (bottom).

Size at first maturity of *D. latifrons* was calculated at 88.1 mm total length. This size is between immature and maturing females with zero growth marks in scales. As the Schnute growth model uses relative age rather than absolute age to determined age at first maturity, we used a derivation of the best case 1 of the Schnute growth in size model with τ_1 in 0.845 (age of females with zero growth mark in scales) and τ_2 6.845. Age was calculated by substituting Yt for total length at first maturity using the equation below:

$$t = \tau_1 - \frac{1}{a} Ln\left[1 - \frac{\left(Y_t{}^b - Y_1{}^b\right)\left(1 - e^{-a(\tau_2 - \tau_1)}\right)}{Y_2{}^b - Y_1{}^b}\right]$$

Age at first maturity was thus calculated to be 0.72 years.

Female chame have a high fecundity. Mature females smaller than 200 mm of total length produced between 39,000 and 170,000 eggs, and females larger than 200 mm produced between 1 and 5 million of eggs (Figure 10). A tendency to to increase the number of eggs per gram of female weight with size was observed, but was not statistically significant (P= 0.09). The mean reproductive potential of chame is 11,910 eggs per g of female weight with a confident interval at 95% of 149,094 and 14,727 eggs.

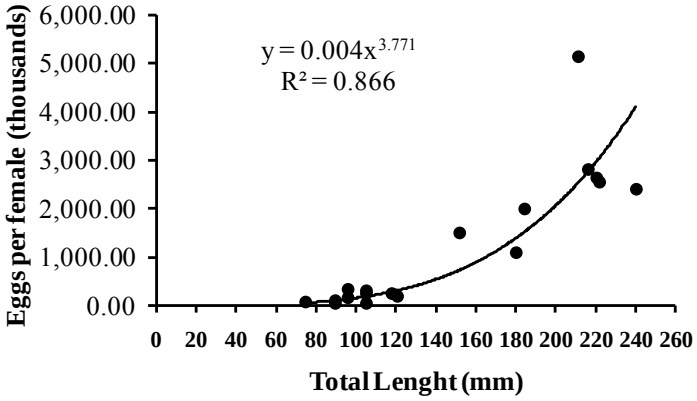


Figure 10. Fecundity for size of female *D. latifrons*.

The catch at age curve showed a decreasing section only, but extreme points no were used to analysis (Figure 11). Mortality rate was estimated at 1.4 year^{-1}.

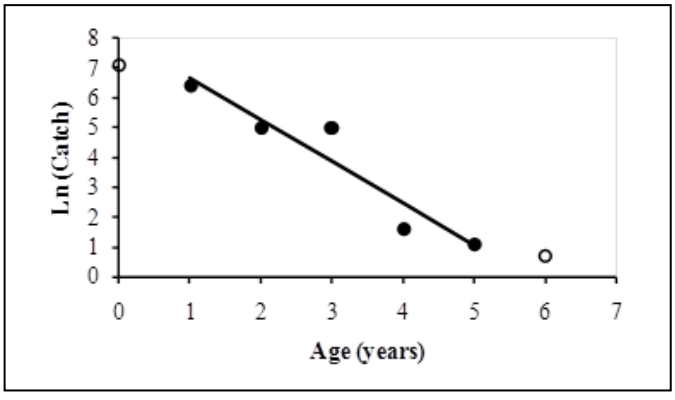


Figure 11. Catch at age curve.

Estimations of natural mortality were derived using four empirical models:

Model	Mortality rate
Rickhter and Efanov (1976)	M= 1.77
Pauly (1980)	M= 0.47
Hewitt and Hoenig (2005)	M = 0.75
Cubillos (2003) after Alverson & Carney (1975)	M = 0.27

DISCUSSION

In most of Mexico this species is not considered a target fisheries species, but is used as bait for fishing and is captured as bycatch in the artisanal shrimp fishery. In the freshwater survey sites, chame was observed to cohabit these areas with other non-predatory species such as tilapia and gobids. In estuaries and costal lagoons, it is subject to predation by catfishes and other species.

Age at first maturity was calculated as 0.72 years but an absolute age of 0.845 for fishes of age 0 group was assumed. If 1 year absolute age for 0 group is assumed, then age at first maturity is 0.88 years and natural mortality rate from Rickhter and Efanov (1976) empiric model would be 1.4, the same as estimated with catch at age curve analysis.

Mortality rates estimated with the empiric models of Pauly (1980) and Cubillos (2003) were lowest. But the problem is that those models use von Bertalanfy parameters which was not the best model describing growth in this study. The Hewitt and Hoenig (2005) model is the simplest and uses only maximumum observed age. In this study, maximumum observed age was 6, and natural mortality was 0.75 $year^{-1}$ but an increment of 1 year of maximumum age could result in a 0.64 $year^{-1}$ rate.

Assuming total mortality of 1.4 $year^{-1}$ (from catch at age curve analysis) and natural mortality rates of 1.4 and 0.75 (from corrected Rickhter and Efanov (1976) and Hewitt and Hoenig (2005)) an "exploitation rate" of 0 to 0.46 is obtained. This means that the "health" of the stock of Chame *D. latifrons* is good, considering the 0.5 exploitation rate as reference point for overexploitation.

D. latifrons is a r-strategist species as revealed by its high fecundity, early age and low size at first maturity. It is associated with floods for dispersal to habitats such as streams, seasonal ponds, estuaries and coastal lagoons. Differences between total and natural mortality rates reveals added mortality from other sources than this life history reveals. Human constructions in the beds of rivers and streams that stop the migratory routes of the species, changes in duration of the dry season, fisheries and shrimp aquaculture were major factors identified that could added this mortality. Despite their high resistance to extreme environmental conditions, a long dry season can reduce the seasonal freshwater lagoons that remain after floods, resulting in total mortality for local stocks as was seen in Mataderos lagoon. Although no fishery is directed at *D.latifrons*, fish mortality most likely results from the shrimp fishery in coastal lagoons. An estuary called "Puyeque" (the common name of chame in the region) is thus named because a lot of *D. latifrons* are caught as bycatch when the shrimp fishery season begins. A lot of chame fishes are disposed of on the shores of the estuaries and coastal lagoons where they decompose without anyone using them as food. *D. latifrons* is common in the ponds and channels of shrimp farms were they compete with shrimp for food. Farmers capture and discard them. This added mortality is very important as revealed in the estimated "exploitation rate". Even without a commercial fishery for chame in the region, the "exploitation rate" is the same order of magnitude for targeted fisheries

species in the region. A management plan is necessary for conservation of the species. Its high fecundity and rapid growth rate could be exploited for aquaculture purposes.

REFERENCES

Alverson D.L. & M.J. Carney. 1975. A graphic review of the growth and decay of population cohorts. Journal du Conseil 36: 133-143.

Castro Rivera, R., G. Aguilar Benítez and J. de la Paz Hernández Girón. 2005. Conversión alimenticia en engordas puras y mixtas de popoyote (*Dormitator latifrons* richardson) en estanques de cemento aquatic, julio-diciembre, número 023 Universidad de Zaragoza, Zaragoza, España pp. 45-52.

Cubillos, L.A. 2003. An approximative relationship to estimate the natural mortality rate in fish stocks. Naga WorldFish Center Quarterly 26: 17-19.

Florencio, A. and M. Serreno. 1981: Algunos aspectos sobre la biologı´a del Chame, *Dormitator latifrons*. Rev. Cienc. Mar. Limnol. 1, 73 –81.

Haddon, M. 2001. 'Modelling and Quantitative Methods in Fisheries. Chapman & Hall/CRC Press: Washington, D.C.

Haz Alvarado. 2002. Producción y exportacion del chame como nueva alternativa comercial del Ecuador. Proyecto de grado, Escuela Superior Politecnica del Litoral. Instituto de Ciencias Humanisticas y Economicas. 167 p.

Hewitt, D. A. and J. M. Hoenig. 2005. Comparison of two approaches for estimating natural mortality based on longevity. U.S. National Marine Fisheries Service Fishery Bulletin 103:433–437.

Montgomery, S. S., C. T. Walsh, M. Haddon, C. L. Kesby and D. D. Johnson. 2010. Using length data in the Schnute Model to describe growth in a metapenaeid from waters off Australia. *Marine and Freshwater Research,* **61**, 1435–1445.

Navarro-Rodríguez, M.C., R. Flores-Vargas, L. F. González-Guevara and M.E. González-Ruelas. 2004. Distribution and abundance of *Dormitator latifrons* (Richardson) larvae (Pisces: Eleotridae) in the natural protected area "Estero El Salado" in Jalisco, Mexico. Biol. Mar. Oceanog. 39(1): 31-36.

Navarro-Rodríguez, M.C., L.F. González-Guevara, R. Flores-Vargas, M.E. González-Ruelas & F.M. Carrillo-González. 2006. Composición y variabilidad del ictioplancton de la laguna El Quelele, Nayarit, México. Biol. Mar. Oceanog. 41(1): 35-43.

Navarro-Rodríguez, M. C., R. Flores-Vargas, L.F. González-Guevara, J. Tellez Lopez and R. Amparan Salcido, 2010. Distribución y abundancia de las larvas de *Dormitator latifrons* (Pisces: Eleotridae) en el estero Boca Negra, Jalisco, México. Ciencia y Mar 2010 XIV (40) 3-9.

Pauly, D. 1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. Journal du Conseil 39: 175-192

Rickhter, V.A. and V.N. Efanov. 1976. On one of the approaches to estimation of natural mortality of fish populations. International Commission of the Northwest Atlantic Fisheries, Research Document 76/VI/8: 1-12.

Rojas-Herrera, A., J. Violante-Gonzalez and D. S. Palacios-Salgado. 2009. Length–weight relationships and seasonality in reproduction of six commercially utilized fish species in the coastal lagoon of Tres Palos (Mexico). J. Appl. Ichthyol. 25 , 234–235.

Consolidation of Native Species Aquaculture in Southeastern Mexico: Continuation of a Selective Breeding Program for Native Cichlids and Snook Aquaculture

Indigenous Species Development/Experiment/09IND05UA

Wilfrido M. Contreras-Sánchez, María J. Contreras-García, Alejandro Mcdonal-Vera, Carlos A. Alvarez-Gonzalez, Ulises Hernández-Vidal, Rafael Martinez-García and Arlette A. Hernández-Franyutti
Universidad Juárez Autónoma de Tabasco
Villahermosa, Tabasco, México

Kevin Fitzsimmons
University of Arizona
Tucson, Arizona, USA

Undergraduate Biology Students:
Maria Jesús Frías Izquierdo and Isabel Ascencio Díaz
Universidad Juárez Autónoma de Tabasco
Villahermosa, Tabasco, México

INTRODUCTION

One of the most important fisheries resources of Mexico is the snook group. Its economic importance is so big that this group of fish is considered among the most precious organisms for coastal and inland fishermen. This pressure over the resource has generated a high exploitation resulting in capture decreasing, especially for large organisms- mainly composed of adult females. Within most of the countries of the range of the snooks, the issue is the same. As a result, researchers from United States, Central America and Brasil have demonstrated their concern to develop actions that ensure the continuity of the management of this resource (Tucker y Campbell, 1988; Taylor et al., 1998; Álvarez-Lajonchere et al., 2002; Cerqueira y Tsuzuki, 2009). In the US, especially in Florida and Texas, sport fishing constitutes a very important economic industry and there have been isolated efforts for artificially reproducing these specie for restocking (Tucker y Campbell, 1988; McMichael et al., 1989; Taylor et al., 1998; Neidig et al., 1999; Skapura et al., 1999 and Main et al., 2009).

The common snook *Centropomus undecimalis* and the fat snook *Centropomus parallelus* are two fish species distributed from the US to Brasil in the Atlantic Ocean. They are euryhaline and at juvenile stage are found in estuaries (Castro-Aguirre et al., 1999). These fish are difficult to reproduce in captivity (Álvarez-Lajonchere y Hernández-Molejón, 2001) thus sometimes it is necessary to collect them and adapt them to captivity (Álvarez-Lajonchere y Hernández-Molejón, 2001). This adaptation in most of the cases result in holding the fish for long time, feeding them with fish which can result in high costs (Alves et al., 2006), so it is necessary to develop strategies that allow the inclusion of feed based on meals available in the market. Having artificial feed available for snook in captivity will allow us to give all nutrients the fish need at sufficient levels, resulting in an increase in growth rate (Roberts, 2002). In addition, the determination of snook gene expression let the generation of valuable information on initial ontogeny in order to understand the identity of the best feed to establish the snook culture.

The snook *C. poeyi,* on the other hand is one of the species of this genus with fewer studies. However, it is known that is endemic of the Gulf of Mexico from Tampico, Tamaulipas to Centla, Tabasco and have catadromous habits –adults are found in fresh waters, mature organisms in estuarine zones and in low river zones-. While juveniles are located in mixed-haline environments and at the adjacent neritic zone (Chávez, 1963).

The development of aquacultural techniques for native species not only can reduce the pressure over wild populations, but can also provide a reliable supply for the development and implementation of working plans for restocking the overexploited stocks. At the same time, a sustainable fishery of native species could contribute to solve the rural emigration problem providing new and better employment and income opportunities. The present project arises from the necessity of continuing the development of research that let us achieve the correct methodologies in order to obtain snook fry from captive broodstock. The design of right feeding during early development will allow us to obtain fingerling survival for the incorporation of aquacultural activities through strategic evaluation.

METHODS AND MATERIALS

Establishment of Common snook (*C. undecimalis*) and fat snook (*C. parallelus*) broodstock groups from wild and hatchery

Objective 1: To obtain broodstock from wild and hatchery-reared snook juveniles
General Experimental Procedure. Among the proposed studies for the present investigation we proposed the creation of two lines of *C. undecimalis* and *C. parallelus;* one initiated from juveniles captured from the wild at the Laguna de Mecoacan and the Gonzalez River. The other line would be obtained from induced spawnings of broodstock keep in captivity since 2008.

Experiment 1a: Origin of the wild organisms. The organisms were captured at the Laguna de Mecoacán, Paraíso, Tabasco, using a mosquito mesh net of 10 m length and 2 m height. Sampling was conducted close to the edge of the lagoon in places were mangrove roots allow the passage of the net and fishermen, 126 juveniles were captured and transferred to the Marine Aquaculture Station (Universidad Juárez Autónoma de Tabasco) for their adaptation to captivity. These fish mere placed in plastic tanks of 1 m^3 capacity containing filtered marine water and provided with constant aeration. After acclimation, the fish were transferred to the Tropical Aquaculture Laboratory at the Biological Division (UJAT) located in Villahermosa. Fish were acclimated to fresh water in a recirculating system composed of 1,300 L plastic tanks and equipped with sand filter and constant aeration.

The feeding regime was: during the first 14 days, snooks were fed with live cichlids (*Cichlasoma Urophthalmus* and *Oreochromis niloticus*) ad libitum; the day 15 and 16 fish were fasted; day 17 and 18 fish were fed ad libitum with chunks of fresh fish; during days 19 and 20, the fish were fasted and during days 21 and 22, fish were fed with pieces of fresh fish. For days 23 and 24, the fish were fasted again and from day 25 onwards the fish were fed with a semi-humid diet two or three times per day. The diet was designed based on fish meal, grounded fish fillet, shrimp meal, fish oil, soy lecithin, a mix of vitamins and minerals, vitamin C, unflavored gelatin, soy milk and sorghum flour, all these ingredients have been used in similar investigation by other authors (2009; Cerqueira and Tsuzuki, 2009).

Samplings were performed monthly in order to determine growth in weight and length; fish were anesthetized with either Methanesulphonate of tricaine (MS 222) or clove oil.

Experiment 1b: Obtaining and maintaining *C. undecimalis* broodstock. Wild organisms adapted for three years to captivity were used**.** Nine females were selected from this stock with sizes ranging from 60 to 91 cm and a weight between 1.896 and 5.290 kg. Eighteen males with an average length of 83 cm and a weight ranging from 806 and 3,886 g. Organisms were maintained at the Marine Aquaculture Station from UJAT in Jalapita, Centla, Tabasco. The adaptation to captivity was performed in 25 m^3 geomembrane tanks. Fish were fed with wild sardines from the Clupeidae family collected with a seine net along the shore, and Breed-M de INVE® feed.

Experimental design: Due to insufficient number of tanks a randomized block design was used (date of replication) with three treatments (0 µg/fish, 100 y 200 µg/fish de GnRH-a). The experiment was replicated three times.

Spawning induction of *C. undecimalis* through GnRH-a implants. In this experiment the method used was from Álvarez-Lajonchere and Hernández-Molejón, 2001. Geomembrane tanks of 4 m diameter connected to a recirculating system were used. Thanks had an eggs collector adapted; consisting of a cylindrical tank of 100 L containing a 400 µm mesh bag. Seawater was used in all tanks with a daily water exchange of 50%. We measured; dissolved Oxygen, temperature (oxygen meter YSI55®) and salinity (refractometer SR6 Vital Sine Premium®). The average value of temperature, DO and salinity during the spawning trials were; 30.65 ± 0.45 ºC, 4.24 ± 0.53 mg/L and 27.5 ± 4.36 ppm, respectively.

Oocyte samples were taken using female's cannulation with a caliber 5 probe and 90 cm in length. Oocytes were measured with a stereoscope (Zeiss®) in order to determine the right diameter for induction (>300 µm). If females had the right oocyte diameter, a female and two males were placed randomly in each tank. Each fish was measured and weighed (with a conventional ichtiometer and a Torrey® balance of 2 g precision). An implant with 0 (control), 100 or 200 µg/fish of GnRH-a hormone (Argent Labs®) was placed in each female. All males from the treatments with hormone were implanted with 100 µg of GnRH-a/fish expecting maturation with just one dose. Only mature males with fluid sperm were used.

Organisms were anesthetized using Methanesulphonate of tricaine (MS 222) and implants were placed in the intra-peritoneal cavity under the pectoral fin with a sterile implants syringe (AVID®). A gentamicine antibiotic cream was applied to avoid infections (Álvarez-Lajonchere and Hernández-Molejón, 2001). The experimental tanks were checked every four hours during three days in order to observe eggs presence. When spawning occurred, eggs were incubated. Eggs were collected by overflow using the egg collector bags, and then were placed in 40 L-plastic buckets with water of the same salinity, temperature and gentle aeration.

Treatments effectiveness evaluation: Treatment effectiveness was evaluated using the occurrence or not of spawning activity. The quality of the spawning was assessed using the number of eggs obtained and the percentage of fertilization. The number of eggs was estimated by volume, taking three samples of 50 mL in different places of the tank. Multiple countings of each sample were performed using 1 mL sub-samples (Álvarez-Lajonchere and Hernández-Molejón, 2001). The percentage of fertilization was evaluated using presence or absence of embryo development analyzing 100 eggs of each experiment; observation was conduced using a stereoscopic microscope (Álvarez-Lajonchere and Hernández-Molejón, 2001).

The present study took into account some parameters described by Álvarez-Lajonchere and Hernández-Molejón (2001) and Hernández-Vidal (2002), in order to determine egg quality considering the following: egg diameter, egg number, fertilization rate, percentage of hatching, and

larvae length. The egg diameter was determined using a sample of 100 eggs from each spawning. They were measured with a ocular micrometer calibrated in a stereoscopic microscope (Zeiss®; Hernández-Vidal, 2002). Pelagic eggs (considered viable) were incubated from each spawning (Álvarez-Lajonchere and Hernández-Molejón, 2001). After this, hatching percentage was evaluated following the same method for determination of fertilization rate. The number of larvae was obtained through direct counting (Hernández-Vidal, 2002). The hatched larvae were maintained in clean containers with the same conditions (Hernández-Vidal, 2002). Percentage of survival was also determined. Larvae length was registered at the point where yolk sac was absorbed.

Statistic analysis: the presence or absence of spawning was recorded and egg diameter analyzed using a covariance analysis, blocking the possible effect of the pseudo-replication (replication date). Hormone concentration was used as the factor of interest. Female weight and oocyte initial diameter were used in the analysis as covariates. The variables fertilization rate and percentage of hatching were analyzed using a Chi-square. There was no data analysis for larvae number and size due to there was just data for one replicate of each treatment. All tests were performed using the software STATGRAPHICS™ V 5.1. The statistic differences were considered using a confidence level of $p< 0.05$.

Experiment 1c: For the spawning induction with *C. parallelus* the same method was used as described for experiment 1 using wild and captivity organisms in 2 m diameter tanks.

RESULTS

Objective 1a: Obtaining and maintaining of a C. undecimalis broodstock from the wild.
According with observations, snooks can be adapted easily to captivity conditions; these organisms did not showed problems ingesting live preys, hold or pieces of fresh feed. With the semi-humid feed the adaptation was effective after fasting days.

The statistics analysis indicates the existence of statistics differences (KW; $p <0.01$) among the different months of sampling for weight and for length. The initial average weight was 26.52 ± 24.59g, while final weight was 137.83 ± 79.40 g (Fig. 1), having a gain of 111.31 g in twelve months of sampling (9.2 g per month). For length the initial average was 14.60 cm and the final average 27.04 cm (Fig. 2). The gain of 12.44 cm (1.03 cm per month)

Experiment 1b: Obtaining and maintaining of a *C. undecimalis* broodstock.
After implantation, *C. undecimalis* broodstock behavior was monitored for two days, observing that spawned fish made a courtship swimming in circles going up to the surface forming a spiral. All fish went back to the bottom and restarted this behavior. Was determined that fish spawned 27 hours after implantation. Spawning was obtained from 2 females of each hormonal treatment tested. Given that only the females treated with hormone spawned (4 of 6), in this analysis only those cases were included. There were no statistical differences (ANOVA; $p = > 0.05$) for the number of eggs produced by female among the treatments 100 and 200 µg/fish (1.98 ± 1.27 and 2.46 ± 0.93 million of eggs, respectively).

Results for the diameter of the fertilized eggs indicated that there is no effect of the factors or from the covariables used in this study (ANCOVA; $p > 0.05$). However, the highest average was obtained with the dose of 200 µg/fish with 746.50 ± 2.12 µm and the lowest average for dose of 100 µg/fish with 681.50 ± 23.50 µm. Te results of the statistics analysis for fertilization indicated that there are statistic differences among treatments (X2; $p > 0.001$), the implants with better results were the 200 µg/fish with 76.84%. By other hand the fish induced with 100 µg/fish had a 60.47% fertilization. Was observed that hatching occurred four hours post fertilization. For this variable, the statistics

analysis indicated high statistics differences (X2; p > 0.001), having a percentage of 100 ± 0.00% hatching for 200 µg/fish treatment and 50 ± 60.10% for the treatment of 100 µg/fish.

From all spawned females, only the larvae from one female from each hormonal treatment survived to the first feeding. 540,000 larvae were obtained for the treatment of 100 µg/fish and 3,119,999 larvae for the dose of 200 µg/fish. The larvae from the high dose measured in average 1.56 ± 0.09 mm and the larvae from the low dose 1.98 ± 0.05 mm.

Experiment 1c: In the case of the stock that would be obtained from captivity broodstock with *C. parallelus* wild and captivity females were induced to spawn. The obtained eggs were not viable.

METHODS AND MATERIALS

Mexican snook (*Centropomus poeyi*) reproduction through GnRH-a implants

Objective 2: to obtain spawning from Mexican snook in captivity

General Experimental Procedure. 14 wild organisms (*C. poeyi)* were collected during an annual reproduction season (June - September). Initially were maintained at the Marine Aquaculture Station in quarantine tanks of 25 m^3. Spermatic activation was carried out under microscopic examination in order to confirm that spawning occurs in marine waters using salinities of 0.15 and 35 ppm. Also intraovarian cannulation were performed to obtain oocytes, its diameter was taken before transported to experimentation tanks. Spawning was induced using GnRH-a implants (Argent Lab). Females were implanted with vehicle (pellet without GnRH-a) 100 µg/fish, ó 200 µg/fish. All males were implanted with 100µg/fish. The floating eggs were collected and transported to a density of 100 eggs/L. The 99.5% of the obtained larvae was released in the spawning sites. Marine water was used (31 ppm; salinity found in the sea at the experiment moment) in all tanks; water exchange was done at 80% daily. The DO, salinity and temperature were 5.3 mg/L, 31 ppm and 27 ºC, respectively. After implantation, spawning tanks were monitored every 2 hours in order to observe eggs/embryo presence.

Statistics analysis: The diameter and number of eggs were analyzed through ANOVA. Eggs quality was evaluated through fertilization and hatching rate; these results will be compared among treatments using a Chi square test with contingency tables.

RESULTS

Objective 2: Obtain Mexican snook spawning in captivity. Cannulation of mature females and males were performed during June and July. However, sexual products were not obtained which was used as an indicator to wait for the next month (August), in which no oocytes were obtained in some cases. However, implants were placed to three females with dose of 100 y 200 µg/female. In the cases were oocytes were obtained these had an average measure of 332.5 µm. Spawning occurred around 29 hours post implantation in three of the implanted females, 4 million eggs were obtained with the females with the high dose. For the low dose the number of eggs was around 2 million eggs, however, these eggs were not viable. The average eggs diameter was 541.29 µm for the high dose and 620.31 µm for the low dose. The fertilization percentage was among 18 and 100% and hatching rate among 29 and 68%. Were obtained between 1 and 3 million of viable larvae. For larvae feeding was used the rotifer *Brachionus plicatilis* and microalgae *Nannochloropsis oculata*. Larvae measured an average of 1.5 mm length.

No statistical analysis was performed due to insufficient fish for the application of a experimental design.

METHODS AND MATERIALS

Identification of native plankton for snook feeding

Objective 3: Identification of native plankton used as feed during snook early development
General Experimental Procedure. Phytoplankton and zooplankton was collected from the common snook spawning zones close to the Gonzalez River. The snook spawning sites were marked and three sampling sites were selected: the first site on the fishermen capture area, the second between the capture sites and the coastal line and the third in front of the coastal line. Samplings were performed for 10 minutes with plankton nets of 20, 64 and 120 µm with a boat of 25 ft length at low speed. The samples were fixed with formaline at 4% and were analyzed under stereoscopic microscope.

RESULTS

To date we have made five monthly samples, finding a great variety of organisms: copepods, cladocerans, chaetognaths, brachyuran, clams, polychaetes, appendicularians, nematodes, luciferase, among others. However, the most abundant was registered for copepods, follow by cladocerans, meanwhile the lowest abundance was registered for caridean, nauplii and eggs (Fig. 3-7).

METHODS AND MATERIALS

Objective 4: Determination of the digestive enzyme gene expression during different life stages of the common snook (*Centropomus undecimalis)*.

General Experimental Procedure. Larvae and juveniles of *C. undecimalis* and *C. parallelus* were collected in quantities that allow obtaining a minimum of 150 mg of stomach, intestine and pancreatic tissue. The sampling was carried out after 12 hours of fasting for larvae and 48 hours for the juveniles and adults. Organisms were euthanized through a cold-water shock. Tissue was collected and washes with distilled water, later were submerged in an Rnasa inhibitor (RNA, ambion, AM7020, Austin, TX, USA) and were storage in liquid nitrogen until processed.

Sampling was performed before first feeding by triplicate from the fertilization (400 embryo, 0 DAH) and from 1, 3, 5 and 12 DAH (400 organisms per day). The embryo an larvae samples were rinsed with distilled water and transferred to Eppendorf tubes containing 1.0-1.5 mL of RNALater and storage at -80 ºC for later process.

RNA extraction. The total RNA from each of the samples from embryo, larvae and the portions of intestine, stomach and pancreas which were storage in RNALater at -80 °C, was isolated according to the method of Valenzuela et al. (2005). The concentration of RNA was estimated using the equation of the Lambert-Beer law after reading in the spectrophotomer (Jenway, Genova Ltd, Felsted, United Kingdom) the absorbance (230 nm) of each of the samples. For knowing the integrity of the nucleic acids an electrophoresis was performed in an agarose gel at 1% using an aliquot of 1 µl of mix sample with 1 µl of running buffer of each of the samples at 100 volts in a electrophoresis chamber Mini Sub Cell GT (Bio-Rad, Hercules, CA, USA) for 30 minutes, the resulting bands were visualized in a documentation system of Molecular Image gel Gel Doc XR+ Imaging System (Bio-Rad, Hercules, CA, USA). Once we evaluated the integrity of the genetic

material of each of the samples the aliquots were made from each of the dissected organs according with the obtained concentration, resulting in a final concentration of 0.5– 1µg/µl of RNA.

Reverse transcription of the Polymerase Chain Reaction (RT-PCR). One microgram of the total RNA of each of the samples of intestine, stomach and pancreas was reversibly transcribed according to the instructions from the kit SuperScript III One-Step RT-PCR System con Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) using the specific primers for the ubiquitin (UB), pancreatic lipase (LIP) y trypsin (TRY) genes in a total volume of 25µl in a real time thermocycler iCycler IQ5 Multicolor real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The nucleotide sequence and the alignment temperature for each enzyme used in this study are detailed in Table 1.

The PCR conditions for the reverse transcription in one step of the enzymes were: 1 cycle of 30 minutes at 55 °C; 1 cycle of 94°C; 30 cycles of 1 minute at 94°C, one minute to the specific temperature for each enzyme, two minutes at 72°C; ten minutes at 72°C. The amplify products were visualized in an agarose gel at 2%, using a stair of 100 pb with marker (Promega, Madison, WI, USA). The observed bands were cropped from the gel and purified using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI, USA) kit. The purified bands were send to the Unidad de Síntesis y Secuenciación from the Instituto de Biotecnología de la UNAM for its sequenced.

The different obtained sequences of digestive enzymes were analyzed using BLAST (www.ncbi.nlm.nih.gov/BLAST) for identification according with reported in the database.

Real Time Polymerase Chain Reaction (qRT-PCR). The total RNA from the common snook larvae from days 0, 1, 3, 5 and 12 after fertilization, which was stored in RNALater, was isolated as mentioned above. The reverse transcription was performed according with the kit Improm-II Reverse Transcriptase (Promega). The experiments of qRT-PCR were carried out in order to observe the RNAm expression during the digestive system development. The specific primers for the ubiquitin (UB), pancreatic lipase (LIP) and trypsin (TRI) (trypsinogen) were designed based on the obtained sequence in this study (Table 2).

β-actin amplification was carried out using specific primers choose from the comparison of different ARNm and represented a very conserved region of nucleotide in order to confirm the expression level of a domestic gen and provide a intern control (Table 2). For this, aliquots of 20µl were taken from the reaction mix, which was prepared with iQ SYBR Green Super Mix (Bio-Rad) and the specific primers (UB5' and UB3' of ubiquitin, LIP5' and LIP3' of pancreatic lipase, TRY5' and TRY3' of trypsin, ACT5' and ACT3' of β-actin for qRT-PCR) for a final concentration of 1µM. The qRT-PCR protocol consisted in one cycle at 95°C for 15 minute follow by 35 cycles of 1 minute at 94°C, one minute at the specific temperature of each gen and 3 minutes at 72°C. The reactions were performed by duplicate and each level of expression was calculated according to the 2- ΔΔCt (Livak y Thomas, 2001) method. The mixture of transcribed genes was made in a real time thermocycler StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

RNA extraction: the total extraction of RNA was performed according with TRIZOL protocol. Later the RNA will be treated with Dnasa I for eliminate the genomic DNA. The reverse transcription will be performed using the commercial kit from Improm II.

RESULTS

PCR amplifications. The amplification by PCR using the primers of Table 1 had as result products of 700, 350 and 200 pb for RTAMI (amylase) in intestine, which were named RTAMI1, RTAMI2 Y RTAMI3 correspondingly, 1200 pb for TLPCA (lipase) in pancreas and 800 pb for TRINA (trypsine) in the intestine (Fig. 8). In the case of TAPSA (aminopeptidase) and PEPNOT (pepsinogen) nothing was observed in the agarose gel.

Sequence analysis. From the five PCR products with their primers send to sequence only three of them were possible of obtain sequence products (Table 3). When doing the sequence analysis on BLAST, was observed that in the case of the sequencing products of RTAMI3, the sequence had a similitude of 90% with the partial pancreatic lipase of the Japanese snapper (*Pagrus major*). In the alienation of the TRINAR sequencing a maximum identity (95%) was found with the trypsinogen of the Blowfish (*Takifugu rubripes*). In table 4 we can observed with more detail the similitude found for RTAMI3, TLPCA y TRINAR en el BLAST.

Genes relative quantification. For the determination of the expression levels of ARNm in the different days of collection of *C. undecimalis,* a relative quantification was performed of PCR-TR (Table 5). For the ubiquitin was observed the gen expression from day 0 with respect to the intestine, expressing 0.32 copies of RNAm, this is that the expression was less than in the intestine. The day 5 (0.76 copies RNAm) was expressed a little more than double than in day 0 although, in less quantity than in the intestine. For days 1, 3 and 12 was not possible to calculate the expression level due to values for Ct en el PCR-TR were not obtained. For trypsinogen was observed the gen expression from day 3 (0.33 copies RNAm) with respect to the intestine and increased for day 5 to 0.47 copies RNAm. No values of Ct from days 0, 1 and 12 in trypsinogen gen were obtained. In the case of the pancreatic lipase we observed that in day 0 there were 8.05 copies greater than in the pancreas.

METHODS AND MATERIALS

Objective 4: Determination of the digestive enzyme gene expression during different life stages of the fat snook (*Centropomus parallelus*).

Five adult fish (150 g mean weight) were captured in the coastal shore of Jalapita, Paraiso, Tabasco, Mexico. Fish were transported to the Laboratorio de Acuicultura Tropical de la DACBIOL-UJAT and maintained in one 2000-L circular plastic tank for 48 h without feeding. To obtained samples of intestine, fish were sacrificed after anesthetized with MS-222, and individual dissection of anterior intestine was obtained. The sampling of the fish was realized to the 7:30 A.M. The intestines were rinsed with distilled water, placed in vials and freeze introducing them immediately in liquid nitrogen. The samples collected, were dissected in cold on a Petri dish sterilized and placed on a cold plate. The fresh, individual and total weight was registered, and they were finally submerged into inhibiting buffer of RNAses (RNA Later, Ambion, AM7020, Austin, TX, USA) for they transport and processing in Biological Research center of the Northwest in La Paz, B.C.S.

Extraction of RNA

The total extraction of RNA took place from the homogenized of the intestines (125 mg of wet tissue), according to the protocol of the reagent of TRIZOL Invitrogen, (Life Technologies SKU# 15596-018, California, USA). Later, the RNA was dealt with DNAse I (Deoxyribonuclease I, Amplification Clay, Invitrogen Cat. No. 18068-015 the USA, Tree-lined avenue, CA, USA) to eliminate the genomic DNA. For the retro-transcription use kit commercial Improm II (Promega, A3800, Wisconsin, USA).

Design of primers
To performed the PCR, the primers used for this study were design for the European sea Dicentrachus labrax (Peres et al. 1998), registered in www.ncbi.nhl.nih.gov (Table 6). In addition electrophoresis was realized in agarose gels (1%) to determine the best temperature for amplification, and bands were stained with ethidium bromide. These bands were recovered using the protocol of kit GENE CLEAN SPIN KIT (BIO 101 INC., Californian, USA) and sent to Macrogen Inc. Korea for their sequencing.

Amplification of cDNA
The amplification of cDNA was realized in a thermocycler Icycler (BIO-RAD ICYCLER, California, USA) with system of temperature gradient, optimizing itself in terms of temperature of alignment and concentration of Mg^{2+}.

The reactions for a PCR were carried out in 50 µl, containing 50 pmol of forward and reverse primers of the trypsin gene, 1 µl of cDNA, 0,5 Or of Taq polymerase (Invitrogen), 100µM of dNTP and 1X of buffer solution (Invitrogen), under the following conditions: 30s to 95°C; 35 cycles of 1 min. to 95°C, 1:30 s to 60°C, 2 min to 72°C; 7 min to 72°C; 4°C ∞.

The purity of cDNA was quantified by means of biophotometer (Eppendorf, 22331), being the standard of reading of double chain (dsDNA) for cDNA higher to 1,6 and lower to 2.1 µg ml^{-1}. The amplification products were separated by electrophoresis in agarose to the 1,5% (A5054 Sigma, Missouri, USA) and later analyzed in a photodocumentator (transiluminator Gel-Pro, **C-62**, CA, USA). The recovery of the DNA was obtained cutting to the bands of interest with a sterile bistoury in the transiluminator and for the purification use the protocol of kit GENE CLEAN SPIN KIT (BIO-RAD 101, CA, USA).

Finally, the amplified segments by PCR were cloned according to the protocol of kit TOPO TA CLONING (Invitrogen n° K4550-40, California, USA) using competent cells of *E. coli* Top 10F' and the plasmid pCR 2,1 like a vector. Once cloned, plasmids of the cells of *E. coli* were obtained using the procedure of kit RPM (BIO 101, California, USA) to be sequence and obtain their homologies with the banks of sequences.

Polymerase chain reaction (PCR)
The PCR of trypsin gene cDNA, was realized according to the protocol suggested by Applied Biosystems user's guide using specific Taqman® probes for the genes and the method of standard curve with plasmids. This was carried out in a thermocycler (Applied Biosystem, Abi Prism® 7000 Sequence Detection System, 4330087, California, USA). The PCR reactions were carried out in plates of 96 wells under the following conditions: 1 cycle to 50°C for 2 min; 1 cycle to 95°C for 10 min; 40 cycles to 95°C for 15 sec and 60°C for 1 min.

A set of sequential dilutions (0, 10, 100, 1000, 10 000, 100 000 and 1 000 000) of the plasmid containing inserted of the trypsin gen at 50 µg ml^{-1} and the 18S rRNA gene (Applied Biosystems), which was used like endogenous control.

Simultaneously, amplification of the trypsin gene of each individual intestine samples were realized (M1D0-M16D30), which were prepared using cDNA, corresponding to every day of the sample, with an approximated concentration of 1000 µg ml^{-1}, TaqMan® Universal PCR Masters Mix (4331348 CA, USA) and a specific Taqman probes (4331348, Custom Taqman (R) Gene Assay Service TRYTG2007-TTD, CA, USA) for trypsin gene for *C. parallelus.* The corresponding calculations were realized on the basis of the protocol *User Bulletin #2 of ABI PRISM 7700 Sequence Detection System.*

Analysis of similarity
Combined to the study of the amplification of the trypsin, an analysis of similarity was realized comparing our sequence with other sequences of fish species. The sequences were obtained from the GenBank/EMBL/DDBJ using Educative Surroundings of "Biology Workbench" on line to perform the blast. The species selected for this analysis were: *Pleurogrammus azonus* (AB441709), *Tribolodon hakonensis* (AB445492), *Salvelinus leucomaenis* (AB447372), *Sparus aurata* (AF316852) *Danio rerio* (AJ297822), *Dicentrarchus labrax* (AJ006882), *Paralabrax maculatofasciatus* (AJ344566), *Oreochromis niloticus* (AY510093 and AY737394), *Oreochromis aureus* (AY510094), *Chelon labrosus* (AY628239), *Symphysodon aequifasciatus* (AY690664), *Cebidichthys violaceus* (AY973822), *Anoplarchus purpurescens* (AY986477), *Xiphister mucosus* (AY986478), *Xiphister atropurpureus* (AY986479), *Spinibarbus sinensis* (DQ839550), *Myxocyprinus asiaticus* (EF493027), *Menidia estor* (FJ859998), *Salmo salar* (X70071, X70073, X70074 and X70075) and *Paranotothenia magellanica* (X82223). The alignment of these sequences allowed calculates the percentage of similarity and the dendrogram.

RESULTS

Amplification of cDNA
The amplification of cDNA from *C. parallelus* trypsin gen gave us a product of 525 pair of bases pair (pb) (Fig. 10). Additionally, in figure 11 we showed the gene sequence and the amino acid sequence (75 amino acids) for the partial trypsin gene of this species.

Analysis of similarity
An alignment of the nucleotides sequences of the trypsin gene in relation to a great variety of fish species was conducted. This analysis revealed that the trypsin gene enough is low conserved with species such as *Sparus aurata* (12.04%), *Chelon labrosus* and *Myxocyprinus asiaticus* (12.10% and 20.08% respectively); on the other hand, the highest similarity percentage was determined with *Myxocyprinus asiaticus* (39.86%) and *Dicentrarchus labrax* (39.40%) (Table 7).
On the basis of the pre-alignment of the sequences, a dendrogram was created, which demonstrates the close relation of the trypsin genes in several fish species, since it can be observed that they come from a common ancestor. With respect to the *C. parallelus* it can be observed that one is in the same group that *S. aurata* (Fig. 12).

DISCUSSION

The study of obtaining a broodstock of common snook suggests that the growth of juveniles from the coast of Tabasco, have a similar growth in captivity similar to reports from Sánchez-Zamora *et al*., (2003) they suggested in their study that this specie have a growth of 0.8 g in a year and our study indicates a growth of 0.7 g per month. For the case of growth in length studies like Aliume (2000) mention that common snook could have an increment of 0.04 cm. Our study suggest a gain in length pretty similar, with data of 1.03 cm/day.

With respect to the reproduction induction of *C. undecimalis* and *C. poeyi* through implants, was possible to obtain spawns and larvae using GnRH-a, with 100 µg/fish with oocytes diameter of 333.00 ± 54.08µm and salinities from 21 ppt in *C. undecimalis.* The spawning obtained in our study match the reproductive season reported for *C. undecimalis* by Taylor et al. (1998) in the coast of Florida and by Perera *et al*. (2008) in the coast of Tabasco. Was also possible to identify that in *C. poeyi* the spawning season is similar for *C. undecimalis* in captivity conditions. This indicate that common snook and Mexican snook populations maintained in captivity completed the oogenesis through a hormonal stimulus. Our results match with other studies of common snook, where they have obtained spawn with viable larvae in fish keep in captivity using implants. However, the

effective dose (50 µg/kg de GnRH-a) varies in the method to apply, which is according to fish weight (Soligo, 2007). In this study, the percentage of fertilization was 6% and survival 7.5%. Skapura et al. (1999) evaluated implants in *C. undecimalis* with dose of 10 µg/kg/day during five days, using a control group and a placebo group. Results indicated that the used of the mGnRH, sGnRH and cGnRH-II implant allow the success in ovulation and Neidig *et al* (1999) determined that the used of Human Chorionic Gonadotropin (GCH) in *C. undecimalis* with dose of 500 IU/kg of weight produced ovulation, good quality eggs and larvae survival. Our results are also similar to the obtained with fat snook (*C. parallelus*) using the same dose (100 y 200 µg/fish) of GnRH-a with the implants technique (Contreras-García, 2011). The percentage of fertilization with 100% in some experimental units and hatching of 10% in some cases, were also similar, in addition successful spawning occurred in *C. poeyi* with results of 100% fertilization.

Cerqueira (2009) with fat snook used LHRHa implants and obtained four consecutive spawning with the same female. That study allowed the observation that the larvae survival until juvenile stage was only of 1%, increasing in later inductions to 30%.

The above suggest that the use of GnRH-a gives a balance stimulus and probably a better integration in the reproductive event with other physiologic functions, affecting direct or indirectly hormone release necessary for the success in final oocytes maturation, the spermiation and the spawning (Zohar y Mylonas, 2001).

In other fish of the Centropomidae family such as *Lates calcarifer* (barramundi), Almendras et al. (1988) obtained multiples spawning using implants of GnRH-a. A female spawned around 7 million eggs with a percentage of fertilization and hatching of 90% in both cases. The larvae survival in this study was 60.8%. Also was observed that only one male was able of fertilize the eggs of a four consecutive spawning from one female. In our study, the percentage of fertilization is also similar to the barramundi data, only for the number of larvae was different being our less. Also, Ibarra-Castro et al. (2009) obtained larvae in barramundi, from lower dose than our dose (50 µg/kg per female and 25 µg/kg per male) with fertilization percentage (90%) also similar to our study.

In other fish, Basaran et al. (2009) increased the success in the reproduction of the drum fish through injections and implants obtaining spawning 70 to 72 hours post injection and 89 to 90 hours post implant. The oocytes diameter obtained with the first technique was 767.5±1.0 µm and 753.5±1.3 µm under the second technique. For our study, the oocytes diameter was similar to the reported for this fish through implants.

Mylonas et al. (2004) used GnRH-a through implants in *Seriola dumerili* (greater amberjack). In this study, spawnings were obtained around 36 hours post implant and 15 days later. The fertilization was around 100%. In the common snook the used of GnRH-a also is effective through implants for the success in reproduction obtaining spawning in similar times to the reported for greater amberjack. Marino et al. (2003) also used implants of D-Ala6, Pro9, NEt]-GnRH (GnRH-a) with dose of 30.5 to 68.3 µg/kg in *Epinephelus marginatus* (dusky grouper). The 85% percentage of the organisms responded to the induction and ovulated between 60 to 238 hours post implant (eggs were obtained with abdominal pressure), while the control group did not mature. The percentage of fertilization was between 48.2% and 52.2%.

Results showed that GnRH-a through implants is an effective method for produce eggs of good quality in greater amberjack in captivity. In the present study, spawned the 66.7% of the females being the spawning times less than the reported for greater amberjack. As well as with the greater amberjack, we could not obtain spawning with the control group therefore the used of GnRH-a is ideal for obtain viable larvae of these fish.

In *C. undecimalis* and *C. poeyi* we obtained similar average in larvae size to those obtained by Peters et al. (1998) they identified that larvae hatched in Florida measured 1.4 to 1.5 mm. The obtained larvae only survived to the fourth day after the absorption of the yolk sac. To this respect, Witenrich et al. (2009) mention that snook larvae mortality can be explain to the lack of adequate prey in the early life stages. In these stages larvae have rudimentary skeletal elements and at the first feeding the larvae have a digestive system little developed, which can restrict its ability to consume preys.

Witenrich et al. (2009) mentions that the mortality of snook larvae is attributed a la carencia de una presa adecuada en las primeras etapas de vida. En estas etapas las larvas presentan elementos esqueléticos rudimentarios y a la primera alimentación las larvas presentan un aparato digestivo poco desarrollado que puede restringir su capacidad para consumir las presas.

Finally, we can mention that the success in the present study is due to mainly to the maintenance of the broodstock for several years under captivity with perfect feeding and handling strategies therefore the used of GnRH-a implants was efficient to allow the completion of the final oocytes maturation with a slowly release of the hormone, even when the oocytes diameter before the treatment application was less to the ideal diameter reported for snooks.

By other hand, in the identification of the native plankton, we found that in the different sampling zones the most abundant organisms were copepods. This will be a probably indicator that snook larvae consume copepods in their first life stages. This abundance increases importantly for the month of May which match with the natural reproduction season of common snook, reported for several authors.

This suggest that even when copepods have sizes from 0.5 to 3 mm snook larvae could be consuming any of the life stages of this crustacean, such as nauplii stages with sizes of 50 µm. Authors like Streble y Krauter (1987), consider these microcrustaceans together with rotifers the most important feed for many organisms for their early life stages. The follow most abundant class in the spawning sites was cladocerans in this study, which indicate that could be the second feed used by larvae in later life stages. Other authors such as Stottrup y Norsker (1997) indicate that the inclusion of copepods in the marine fish larvae culture sustain a normal development and a good larval growth.

Payne y Rippingale (2000) mention that copepods constitute the natural feed of the marine species larvae and represent a high content of the essential fatty acids and antioxidants. All of the above reinforce the probability of snook larvae are feeding of these organisms either in their nauplius or copepodite stages.

As the study of the gene expression, we could identify three of them which act during the initial ontogeny of the common snook; ubiquitin, trypsinogen and pancreatic lipase. This study give new information, due to this is the first work where digestive enzymes sequencing and PCR-TR for the specie *C. undecimalis.*
Given that there is no knowledge of the common snook genome the β-actin was selected as the normalize gene for the experiments with PCR-TR considering the reported for other species (Darias et al., 2006; Murray et al., 2004; Oku et al., 2006). However, according with the observations in our study (Fig. 9) the β-actin was not constant thus exist changes in the expression in all the sampled days, suggesting that it is necessary to explore new possibilities of reference genes for future studies with *C. undecimalis.*

According with other studies (Murray et al., 2004) the trypsinogen is detected since day 5. The levels of ARNm not only are influenced by the regulation of the gene expression, there are more

conditions such as: nutrition, size differences and the tissue components which could influence the ARNm levels from the gene working on (Hibbeler et al., 2008) thus it is important to keep investigating the effects of feeding or the age in the gene expression in this specie. The activity of the pancreatic lipase is not only regulated at the transcriptional level but also at the posttranscriptional level (Birk and Brannon, 2004).

CONCLUSIONS

The adaptations to captivity conditions in *C. undecimalis* is factible, using as feed semihumid balance diets

The use of GnRH-a through implants in common snook *C. undecimalis* and Mexican snook *C. poeyi* allow the oocytes maturation induction and generate spawning in captivity.

Besides, viable larvae were obtained from dose of 100 µg per female independently of its weight. The spawning could be obtained in salinities of 21 ppt for the common snook; a value under the reported in the literature for this specie and from 30 ppt for the Mexican snook.

The use of life feeds such as copepods could be viable for the survival of the snook larvae.

REFERENCES

Aliaume, C., Zerbi, A., Joyeux, J. C. and Miller, J. M. 2002. Growth of juvenile *Centropomus undecimalis* in a tropical island. Environmental Biology of Fishes 59: 299–308.

Almendras, J. M., Duenas, C., Nacario, J., Sherwood, N. M. and Crim, W. L. 1988. Sustained hormone release. III. Use of gonadotropin releasing hormone analogues to induce multiple spawnings in sea bass, *Lates calcarifer*. Aquaculture 74: 97-111.

Álvarez-Lajonchere, L., Cerqueira, R. V., Reis M. 2002. Desarrollo embrionario y primeros estudios larvales del robalo chucumite, *Centropomus parallelus* Poey (Pisces, Centropomidae) con interés para su cultivo. Hidrobiológica, 132 (002): 89-99.

Álvarez-Lajonchere L and Hernández-Molejón, O. G. 2001. Producción de juveniles de peces estuarinos para un centro en América Latina y el Caribe: Diseño, operación y tecnologías. The World Aquaculture Society, Baton Rouge, LA, 424 pp.

Alves, T. T., Cerqueira, R. V. and Brown, J. A. 2006. Early weaning of fat snook (*Centropomus parallelus* Poey 1864) larvae. Aquaculture 253 (2006) 334– 342.

Araújo, J. and Cerqueira, V. R. 2005. Influência da salinidade na incubação de ovos do robalo-peva (*Centropomus parallelus* Poey, 1860). Maringá, 72: (1), 85-89.

Başaran, F., Muhtaroğlu, C. G., Özden, O. and Özkızılcık S. 2009. Spawning behaviour of shi drum (*Umbrina cirrosa*) after hormone administration. Journal of FisheriesSciences.com. 3(2): 124-133.

Birk, R.Z., Brannon, P.M., 2004. Regulation of pancreatic lipase by dietary medium chain triglycerides in the weanling rat. Pediatr. Res. 55, 921-926.

Caballero-Chávez, V. 2009. Talla de primera madurez y composición de las capturas del robalo blanco en sur de Campeche. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

Castro-Aguirre. 1999.

Cerqueira, R. V. 2009. Spawning and larviculture of the fat snook (*Centropomus parallelus*) and the common snook (*Centropomus undecimalis*) in Brazil. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

Cerqueira, V. R. and Canarín, M. 2008. Multiple spawning of the fast snook *Centropomus parallelus* using different dosage of LHRH. Cybium 2008: 32(2) suppl.: 330-331.

Cerqueira, V. R., Mioso, R. and Canarin, M. 2005. Indução de desova com fertilização natural e artificial e incubação de ovos do robalo-peva (*Centropomus parallelus*). Atlântica, Rio Grande 27 (1): 31-38.

Cerqueira, V. R. and Tsuzuki, M. Y. 2009. A review of spawning induction, larviculture, and juvenile rearing of the fat snook, *Centropomus parallelus*. Fish Physiol Biochem 35:17–28.

Chávez, H. R. 1963. Contribución al conocimiento de la Biología de los robalos, Chucumite y Constantino (*Centropomus undecimalis*) del Estado de Veracruz, México. Ciencia 22 (5): 141-161.

Contreras-García, M. de J. 2011. Inducción de la reproducción en *Centropomus undecimalis y Centropomus parallelus* bajo condiciones de cautiverio empleando inyecciones e implantes de GnRH-a. Tesis de Maestría. Universidad Juárez Autónoma de Tabasco. 103p.

Darias, M.J., Murria, H.M., Gallant, J.W., Astola, A., Douglas, S.E., Yufera, M., Martínez-Rodríguez, G., 2006.Characterization of a partial a-amylase clone from red porgy (*Pagrus pagrus*): Expression during larval development. Comp. Biochem. Physiol., Part B 143, 209-218.

Ferraz, M. E., Cerqueira, R.V., Álvarez-Lajonchere, L. and Candido, S. 2002. Inducao da desova do robalo peva, *Centropomus parallelus*, a través de injecao e implante de LHRHa. Boletim do Instituto de Pesca, Sao Paulo 28 (2): 125-133.

Garduño-Dionate, M., Lorán-Nuñez, R. M., Martínez-Isunza, F. R. and Márquez-García E. 2009. Fecundidad de robalo blanco, *Centropomus undecimalis* en la costa de Ciudad del Carmen, Campeche. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

Godinho, H. M., Da Silva-Serralheiro, P. C., De Medeiros-Ferraz, E., Marcondes-Pimentel, C. M., Da Rocha-Oliveira, I. and De Paiva, P. 2000. Reprodução induzida em robalo *Centropomus parallelus* Poey, 1860. Brazilian Journal of Veterinary Research and Animal Science 37 (1): 37-42.

Gómez-Díaz-Durán, L. M. 2002. Inducción a la maduración y desove del robalo blanco *Centropomus undecimalis* (Bloch, 1972) (Pisces: Centropomidae) mediante la aplicación coriónica humana y una análoga de la hormona liberadora de la hormona luteinizante. Tesis doctoral. Universidad Nacional Autónoma de México, Facultad de Ciencias, México, D. F. 70 p.

Grier, H. 2000. Ovarian germinal epithelium and folliculogenesis in the common snook, *Centropomus undecimalis* (Teleostei: Centropomidae). Journal of Morphology 243: 265-281.

Grier, H. J. and Taylor, R. G. 1998. Testicular maturation and regression in common snook. Journal of Fish Biology 53 (3): 521-442.

Hernández-Vidal, U. 2002. Identificación del sexo y evaluación de la inducción hormonal en el pejelagarto (*Atractosteus tropicus*). Tesis de Maestría. Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Monterrey, Nuevo León, México 83 p.

Hibbeler, S., Scharsack, J.P., Becker S., 2008. Housekeeping genes for quantitatitative expression studies in the thre-spinned stickleback *Gasterosteus aculeatus*. BMC Mol Biol. 9:18.

Holt, G. J. and Kline, R. 2009. Culturing Texas snook: What we have learned so far. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

Ibarra-Castro L, Shipp, G. and Alvarez-Lajonchère L. 2009. Culture of the Asian snook (*Lates calcarifer*, Bloch) in Australia. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

Main, K., Yanes-Roca, C. and Rhody, N. 2009. Status and challenges in larval rearing and fingerling aquaculture of common snook (*Centropomus undecimalis*) in Florida. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

McMichael, R. H. Jr., Peters, K. M. and Parsons, G. R. 1989. Early life history of the snook *Centropomus undecimalis*, in Tampa Bay, Florida. Northern Gulf Science 10 (2): 113-125.

Marino, G., Panini, E., Longobardi, A., Mandich, A., Finoia, M. G. Zohar, Y. and Mylonas, C. C. 2003. Induction of ovulation in captive-reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834), with a sustained-release GnRHa implant. Aquaculture 219: 841–858.

Murray, H.M., Perez-Casanova, J.C., Gallant, J.W., Johnson, S.C., Douglas, S.E., 2004. Trypsinogen expression during the development of the exocrine pancreas in winter flounder (*Pleuronectes americanus*). Comp. Biochem. Physiol., A. 138, 53-59.

Mylonas , C., C., Papandroulakisa, N., Smboukisb, A. Papadakia, M. and Divanacha, P. 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture 237: 141–154.

Neidig, C. L., Skapura, D. P, Grier, .H. J. and Sprinkel, J. M. 1999. A preliminary study- A comparison of doses of Human Chorionic Gonadotropin(HGC) on ovulation, eggs quality and larval survival in common snook, *Centropomus undecimalis* (Bloch) In: Proceedings of the Sixth International Symposium on Reproductive Physiology of Fish; 1999 4-9 Jun; Bergen, Norway; p 429.

Oku, H., Koizumi, N., Okumura, T., Kobayashi, T., Umino, T., 2006. Molecular characterization of lipoproteine lipase, hepatic lipase and pancreatic lipase genes: Effects of fasting and refeeding on their gene expression in red sea bream *Pagrus major*. Comp. Biochem. Physiol., B. 145, 168-178.

Payne, M. F. and Rippingale, R. J. 2000. Evaluation of diets for cultura of the calanoid copepod *Gladioferens imparipies*. Aquac 187:85-96.

Perera-García, M. A., Mendoza-Carranza M. and Páramo-Delgadillo, S. 2008. Dinámica reproductiva y poblacional del robalo, *Centropomus undecimalis* (Perciformes: Centropomidae), en Barra San Pedro, Centla, México. Universidad y Ciencia, Trópico Húmedo 24(1): 49-59.

Perera-García M. A., Mendoza-Carranza, M., Contreras-Sánchez, W., Pérez-Sánchez, E. Huerta-Ortiz, M. y Páramo-Delgadillo, Salomón. 2009. Biología reproductiva y poblacional del robalo blanco, *Centropomus undecimalis*, en dos ambientes tropicales,Tabasco, México. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. p26.

Peters, K. M, Mathenson, R. E. Jr. and Taylor, R. G. 1998. Reproduction and early life history of common snook, *Centropomus undecimalis* (Bloch), in Florida. Bulletin of Marine Science 62 (2): 509-529.

Reis, M. D. y Cerqueira, R. V. 2003. Indução de desova do robalo-peva *Centropomus parallelus* Poey 1860, com diferentes doses de LHRHa. Maringá, V. 25 (1): 53-59.

Roberts, R. 2002. Nutritional pathology, In: Fish Nutrition, p 453-504 (Edit.: Halven, E. J. y Hardy, W. R.824P.

Sánchez-Zamora, A., García Galano, T., Gómez-Díaz-Duran., L. M. y Suárez Bautista, J. 1999. Cultivo de robalo *Centropomus undecimalis*: Aspectos reproductivos y crecimiento de juveniles. Memorias de la IV Reunión Nacional de Redes de Investigación en Acuacultura. V reproducción y genética. Octubre 1999 Cuernavaca, Morelos, México. 19-21.

Sánchez-Zamora, A., Rosas, C., Durruty, C., Suárez, J. 2003. Reproducción en cautiverio de robalo: Una necesidad inaplazable en el sureste mexicano. Panorama Acuícola, Magazine 2003; 7:24-5.

Sánchez-Zamora, A. 2009. Situación actual de la reproducción del robalo blanco en cautiverio, en la UMDI, Sisal, Yucatán. In: Segundo Simposio Internacional sobre Biología y Cultivo de robalos. Villahermosa, Tabasco, México. Aquafish. Collaborative Research Support Program. 26.

Skapura, D. P., Grier, H. J., Neidig, C. L., Sherwood, N. M., Rivier, J. E. and Taylor, R. G. 1999. Induction of ovulation in common snook, *Centropomus undecimalis*, using gonadotropin-releasing hormones. In: Proceedings of the Sixth International Symposium on Reproductive Physiology of Fish; 1999 Jun 4-9; Bergen, Norway; p 430.

Soligo, A. T. 2007. Primeiras experiencias com a reproducao, larvicultura e desmame do robalo-flecha, *Centropomus undecimalis* no Brazil. Universidad Federal de Santa Catarina. Programa de Pós-Graduacao em Aquicultura, Florianópolis, 40 p.

Stottrup, J. G. and Norsker, N. H. 1997. Production and use of copepods in marine fish larviculture. Aquaculture 155:231-247.

Streble, H., Krauter, D. 1987. Atlas de los microorganismos de agua dulce, la vida en una gota de agua. Omega, Barcelona.

Taylor, R. G., Grier, H. J. and Whittington, J. A. 1998. Spawning rhythms of common snook in Florida. Journal of Fish Biology 53: 502-520.

Tonini, W. C. T., Braga L. G. T. and Vila Nova D. L. D. 2007. Dieta de juvenis do robalo *Centropomus parallelus* POEY, 1860. No Sul da Bahia, Brazil. B. Inst. Pesca, São Paulo, 33(1): 85 - 91.

Tsuzuki, M. Y., Cardoso, F. R. and Cerqueira, V. R. 2008. Growth of juvenile fat snook *Centropomus parallelus* in cages at three stocking densities. B. Inst. Pesca, São Paulo, 34(2): 319 – 324.

Tucker, W. J. and Campbell, W. S. 1988. Spawning season of common snook along the east central Florida coast. Florida Scientist, 51 (1): 2-6.

Wittenrich, M. L. Rhody, R. N., Turingan, R. G. and Main, K. L. 2009. Coupling osteological development of the feeding apparatus with feeding performance in common snook, *Centropomus undecimalis*, larvae: Identifying morphological constraints to feeding. Aquaculture 294: 221–227.

Zohar, Y. and Mylonas, C. 2001. Endocrine Manipulations of spawning in cultured fish: From hormones to genes. Aquaculture 197: 99-136.

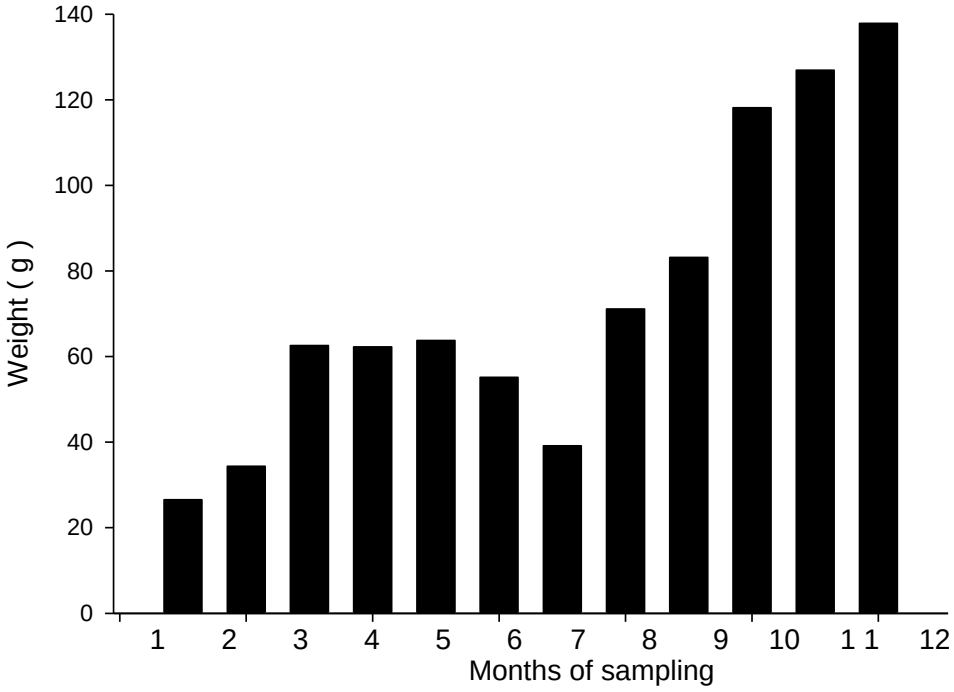


Figure 1. Average weight of common snook juveniles in captivity during a year.

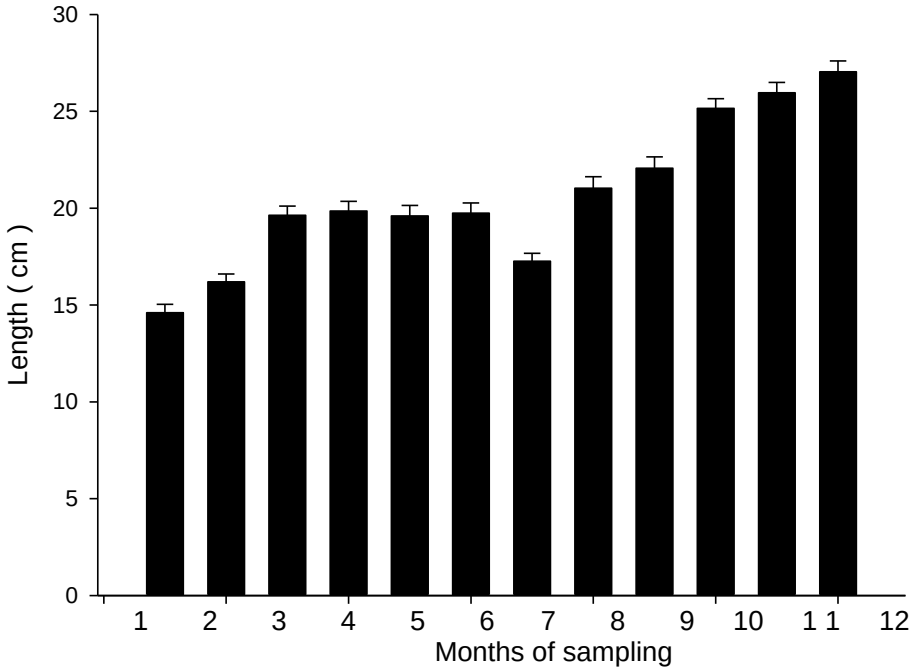


Figure 2. Average total length of common snook juveniles in captivity during a year.

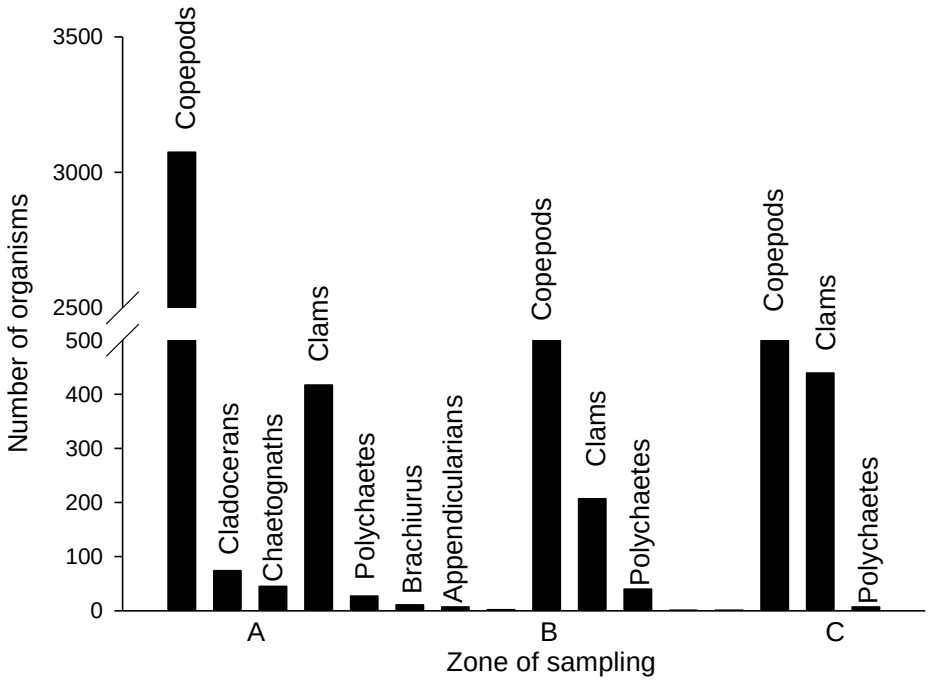


Figure 3. Abundance of groups of zooplankton in April in the coast of Centla, Tabasco.

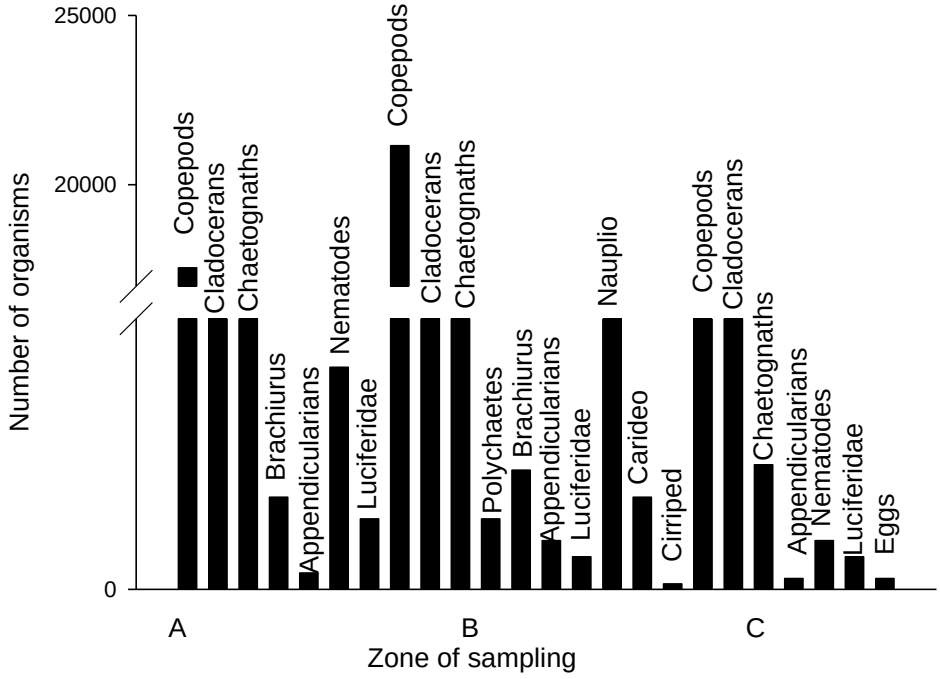


Figure 4. Abundance of groups of zooplankton in May in the coast of Centla,Tabasco.

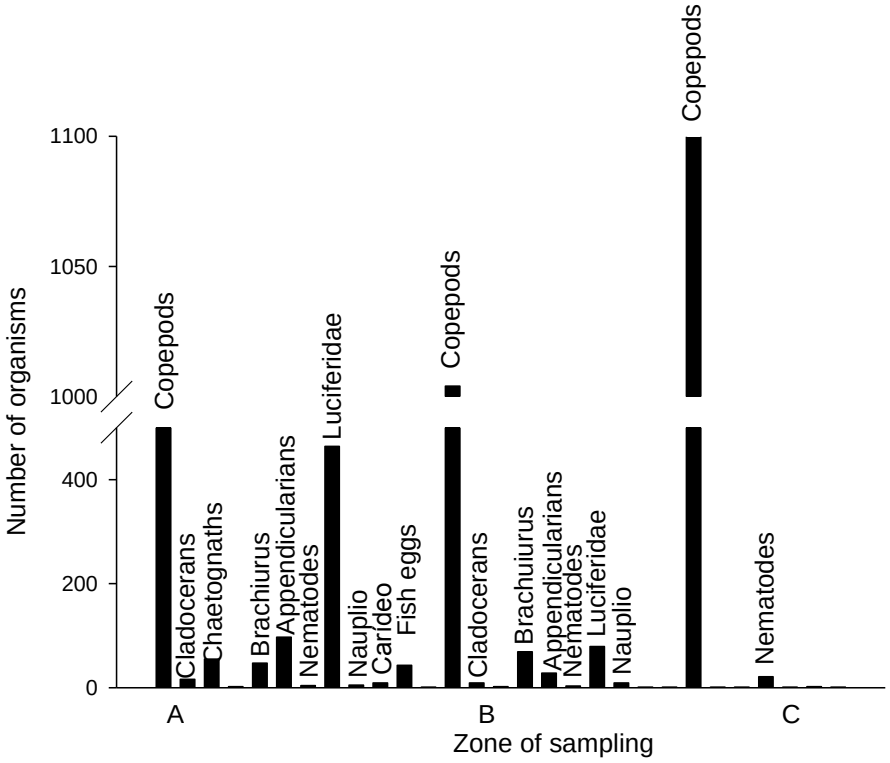


Figure 5. Abundance of groups of zooplankton in June in the coast of Centla, Tabasco.

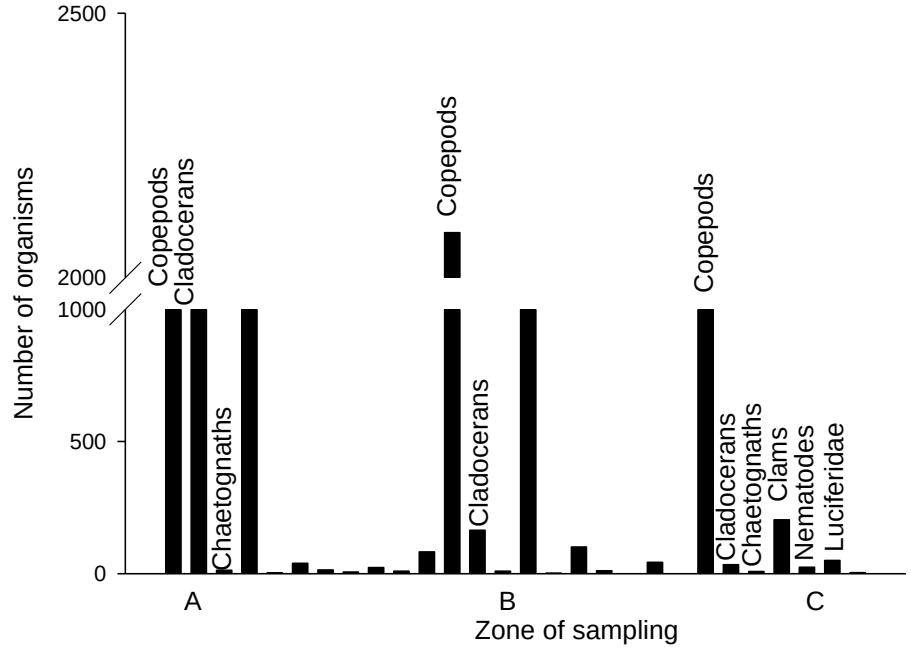


Figure 6. Abundance of groups of zooplankton in July in the coast of Centla, Tabasco.

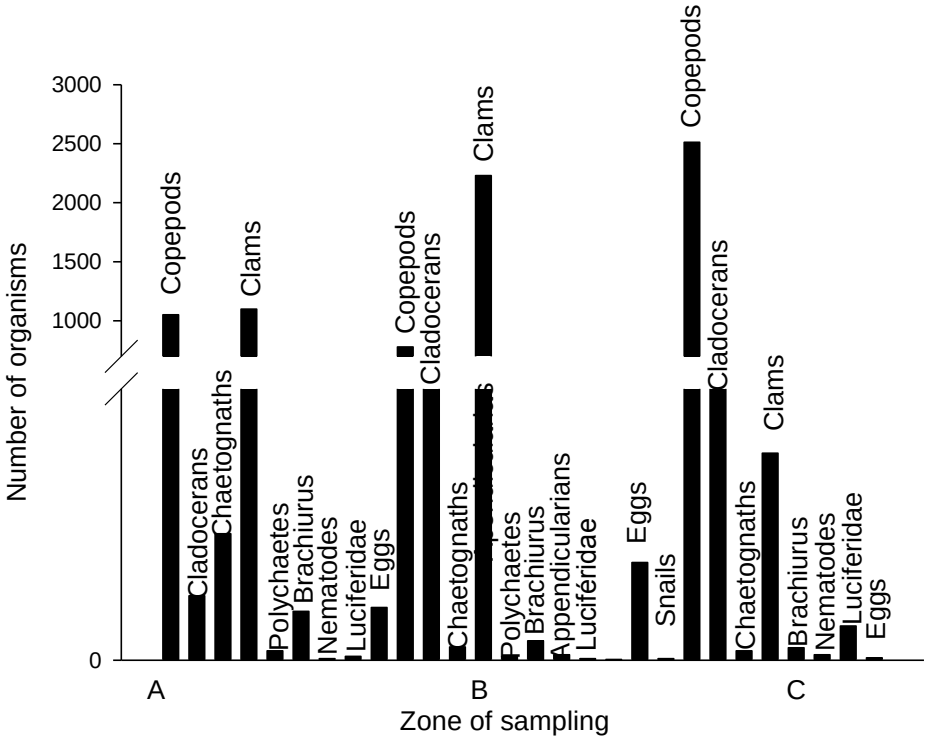


Figure 7. Abundance of groups of zooplankton in August in the coast of Centla, Tabasco.

Table 1. Sequence of primers and alignment temperature of RTAMI (amylase), TAPSA (aminopeptidase), TLPCA (lipase), PEPNOT (pepsynogen) and TRINA (trypsine) used in the reactions of Reverse Transcription-PCR

Primer	Nucleotides sequence (5'→3')	Alignment Temperature (°C)
RT-PCR RTAMI		
RTAMI-F	TTCATATTGGCGTTAGTCCT	59.2
RTAMI-R	TTACAATTTGGAGTCCAAGAC	
RT-PCR TAPSA		
TAPSA-F	TACATCAGTAAAGCTGTCGG	60.7
TAPSA-R	AAGGCTCTCATGACCAAGAC	
RT-PCR TLPCA		
TLPCA-F	AGAAGAACCGCTACTACCAG	61
TLPCA-R	GATCTCATTCTCCTCCACCT	
RT-PCR PEPNOT		
PEPNOT-F	GAGTGCCTCCATAAGATTCC	59.3
PEPNOT-R	ACCAATGTACTTAGACTGGG	
RT-PCR TRINA		
TRINA-F	**TCTCTTGTATTCGTTCTGCT**	**60**
TRINA-R	**AGAGGATGATGGAAGAAAGG**	

Table 2. Primers sequence and alignment temperature of RTUbi (Ubiquitine), RTLip (lipase) and RTTri (tripsynogen) which were used in the PCR-TR reactions of snook larvae

Primer	Nucleotides sequence (5'→3')	Alignment Temperature (°C)
PCR-TRUbi		
TRUbi5'	GCAACACACCTGACCTGAGG	55
TRUbi3'	CGTCCTGCTGATTGTATCCC	
PCR-TRLip		
TRLip5'	TGATGCCTGCAATCACGTCC	59
TRLip3'	TTAGCCACTGGGCCATCAGG	
PCR-TRTri		
TRTri5'	GCTCCACTGCTGACAGGAAC	55
TRTri3'	CAGAGTCACCCTGGCAAGAG	
PCR-TRAct		
TRAct5'	**CGCGACCTCACAGACTACCT**	**59/55[1]**
TRAct3'	**GATTCCGCAGGACTCCATAC**	

[1] Alignment Temperature in the PCR-RT for the actine changed according with the studied gen

Table 3. Results from the sequence of the send enzymes to the Instituto de Biotecnología de la UNAM

Enzyme	# de bp	
	Forward	Reverse
RTAMI1	---	---
RTAMI2	---	---
RTAMI3	186	192
TLPCA	870	870
TRINA	---	519

Table 4. Results from the sequence alignment of RTAMI3, TLPCA and TRINA-R in the BLAST

Organism	e Value	Matching percentage
RTAMI3		
Salmo salar ARNm for the clon ssal-rgf-520-156 of the hydrolase ubiquitin carboxiterminal 2, cds pseudogene	5e-27	85
PREDICTO: *Danio rerio* ARNm similar for specific ubiquitin of peptidasa 2	1e-16	79
Oryzias melastigma ARNm for ubiquitin specific of peptidase 2, cds partial	6e-13	88
TLPCA		
Pagrus major ARNm for pancreatic lipase, cds partial	0.0	86
Epinephelus coioides ARNm of colipase-dependt of pancreatic lipase, cds complete	0.0	85
TSA: Hippoglossus hippoglossus all_halibut.1371.C1 mRNA sequence	3e-158	88
TRINA-R		
Takifugu rubripes ARNm for trypsinogen, cds partial	0.0	95
Siniperca chuatsi ARNm for trypsinogen 1, cds complete	0.0	94
Solea senegalensis ARNm for trypsinogen 1b, cds complete	0.0	94
Pleurogrammus azonus ARNm for trypsine, cds complete	0.0	94
Solea senegalensis ARNm for trypsinogen 1c, cds complete	0.0	94
Sparus aurata ARNm for trypsinogen, cds complete	0.0	94
Paralichthys olivaceus ARNm for trypsinogen 2, cds partial	0.0	94
Sparus aurata ARNm precursor for trypsinogen II, cds complete	0.0	94
Dissostichus mawsoni ARNm of the clon 25 for the trypsogen precursor, cds complete	0.0	93
Solea senegalensis ARNm for the trypsinogen 1a, cds complete	0.0	93
Tautogolabrus adspersus ARNm for retripsynogen, cds complete	0.0	93
P.magellanica ARNm for trypsine	0.0	93
Paralichthys olivaceus ARNm for trypsinogen 1, cds complete	0.0	92
Epinephelus coioides ARNm for trypsinogen 1a, cds partial	0.0	91

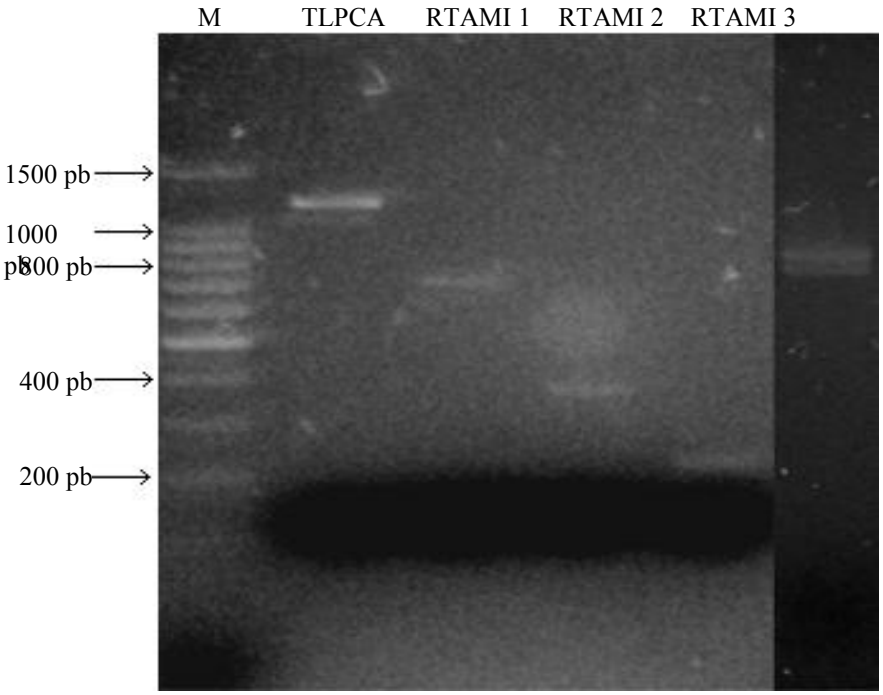


Figure 8. Amplified products for the Lipase (TLPCA), Amilase 1 (RTAMI1), Amilase 2 (RTAMI2), Amilase 3 (RTAMI3) and Trypsine (TRINA) of *C. undecimalis*. Marker (M).

Table 5. Relative expressions of the ubiquitine, trypsinogen and pancreatic lipase in the days of results of Ct for common snook.

Gene	$2^{-\Delta\Delta Ct}$	Control
Ubiquitin day 0	0.32	Intestine
Ubiquitin day 5	0.76	Intestine
Trypsinogen day 3	0.33	Intestine
Trypsinogen day 5	0.47	Intestine
Pancreatic lipase day 0	8.05	Pancreas

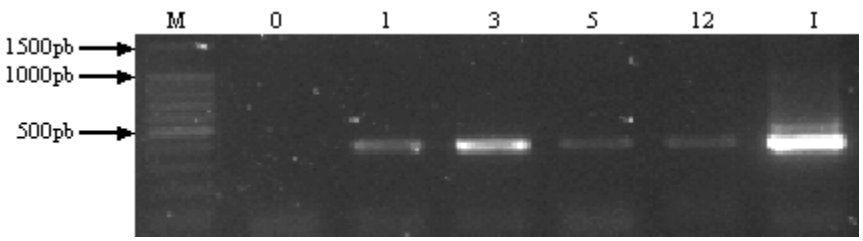


Figure 9. Agarose gel electrophoresis at 2% of the PCR-TR products of the actine gen of common snook (*C. undecimalis*). Larvae age (post fertilization) is indicated above each well. Intestine (I) and Marker (M).

Table 6. Sequence of degenerate primers of trypsin of *Dicentrarchus labrax* (Péres et al. 1998) and temperature of amplification.

Primers	Forward	Reverse	Temperature of amplification ºC
Trypsin	CAggTgTTCTgAAC	CCC (Ag) gACACAACACCT	60

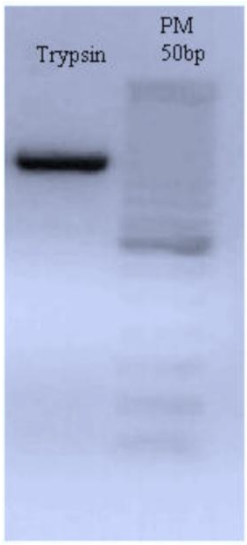


Figure 10. Gel of Agarose in which is the band of trypsin with 525 bp of the intestine of *C. parallelus* adults. PM: Marker of molecular weight of 50pb.

Figure 11. Partial sequence of *C. parallelus* trypsin gene.

Table 7. Percentage of similarity in comparison with *C. parallelus* trypsin gene.

Species	Percentage of similarity
Myxocyprinus asiaticus	39,86
Dicentrarchus labrax	39,40
Cebidichthys violaceus	39,21
Paralabrax maculofasciatus	38,34
Xiphister mucosus	35,85
Anoplarchus purpurencens	35,59
Menidia estor	34,68
Symphysodon aequifasciatus	34,00
Oreochromis niloticus	26,18
Danio rerio	26,04
Paranotothenia magellanica	24,67
Salmo salar	24,62
Salmo salar	24,17
Salmo salar	23,52
Tribolodon hakonensis	23,50
Salvelinus leucomaenis	23,38
Salmo salar	23,36
Oreochromis aureus	23,31
Oreochromis niloticus	23,18
Pleurogrammus azonus	23,08
Spinibarbus sinensis	21,81
Myxocyprinus asiaticus	20,08
Chelon labrosus	14,10
Sparus aurata	12,04

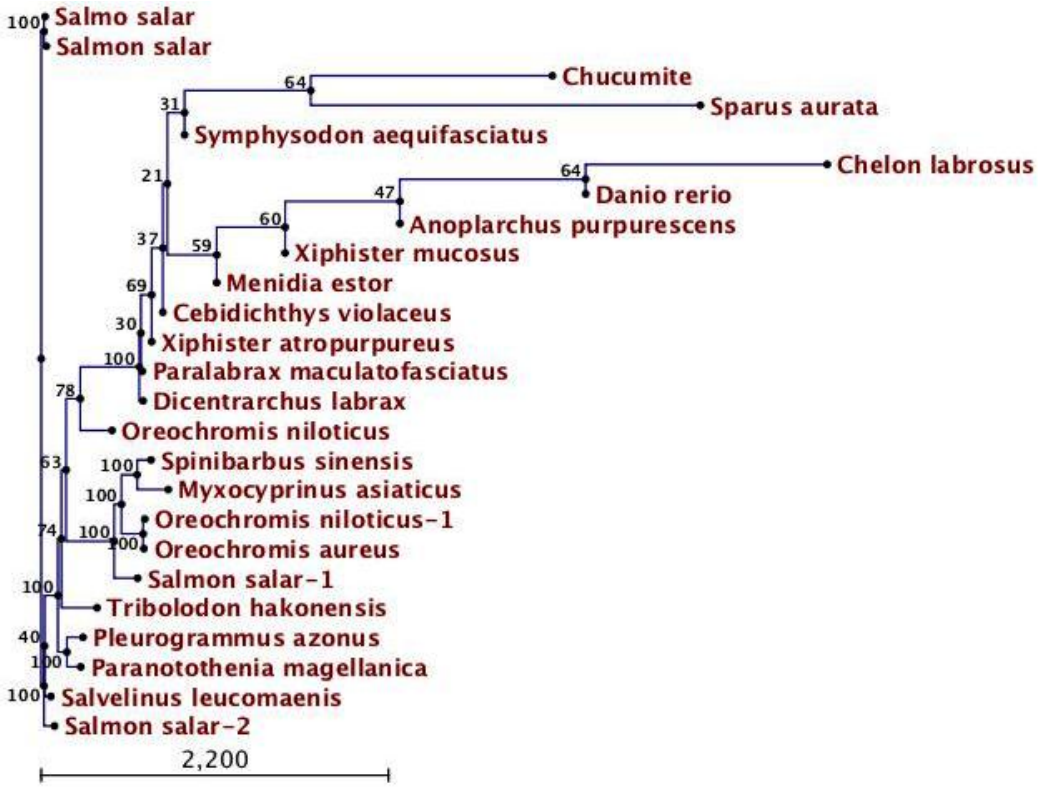


Figure 12. Dendrogram of the alignment of the similarity analysis, where is the relation of the trypsin gene in different fish species.

Development and Diversification of Species for Aquaculture in Ghana

Indigenous Species Development/Experiment/09IND06PU

Emmanuel A. Frimpong
Virginia Polytechnic Institute and State University
Blacksburg, Virginia, USA

Nelson W. Agbo and Stephen Amisah
Kwame Nkrumah University of Science and Technology
Kumasi, Ghana

Graduate Students:
Gifty Anane-Taabeah
Virginia Polytechnic Institute and State University

Ethel Tettey and Peter Akpaglo
Kwame Nkrumah University of Science and Technology

EXECUTIVE SUMMARY

Sub-Saharan Africa has abundant land and water resources, but these have not been tapped to increase aquaculture production significantly in global terms. Recent analyses of Sub-Saharan Africa aquaculture have noted a relative lack of public sector research and development attention to alternative culture systems (e.g., cage culture) in Africa and recommended increased attention to alternative production systems while striving to increase intensity and production from the traditional earthen ponds. Likewise, the analyses identified progress made in Nigeria and Egypt in the production of species other than the tilapias as dictated by local demands for those alternative species, leading to their recommendations for expansion of production of high-demand indigenous species for niche markets. As a business model, diversification of species and systems provides a safety net and access to new markets for investors. This project involved complementary investigations of the opportunities and challenges to the adoption of cage culture as an alternative production system in Ghana, experimental studies of nutritional requirements and a market survey of indigenous, non-traditional aquaculture species with potential for development.

Experiments on optimal dietary protein of *Chrysichthys nigrodigitatus* (substituted for *C. maurus*) revealed that best growth of fingerlings was achieved when the diet contained 42.9% crude protein. In a parallel study with juvenile *Heterotis niloticus* the optimal dietary crude protein level was estimated to be at least 32.1%. These results are within the range of available data for similar species and the studies constitute some of the first of their kind for these specific species. This report also provides a brief review of the natural food habits, habitat requirements, and distribution of the *C. nigrodigitatus* and *H. niloticus*.

Because of its refusal to accept pelleted feed in initial laboratory trials, experimental study of *Parachanna obscura* dietary protein requirements was not feasible. A more in-depth study of the distribution and natural food and habitat requirement and acceptability as a potential aquaculture species in Ghana was completed. The survey of market potential and distribution trends of *Parachanna obscura* showed that the species has widespread occurrence and popularity as food in the four regions studied but with seasonal supply and not clearly sustainable harvest methods. Aquaculture will be a useful intervention for consistent supply and to protect reproductive populations in the wild.

These investigations have produced valuable insight not previously available to farmers and the private sector of Ghana, the Fisheries Commission of Ghana and other relevant government institutions. We expect these results to contribute to diversification and rapid acceleration of aquaculture development in Ghana and the sub-region. A comprehensive Strength Weaknesses Opportunities and Threats (SWOT) analysis of the aquaculture industry in Ghana is underway utilizing lessons learned from past, the current, and ongoing AquaFish CRSP projects. This analysis is being undertaken with input from the Fisheries Commission of Ghana.

Ecology And Effects Of Dietary Protein Levels On Growth Performance Of Claroteid Catfish, *Chrysichthys Nigrodigitatus,* Fingerlings

ABSTRACT

Chrysichthys nigrodigitatus is a relatively new aquaculture species in Ghana and knowledge about its culture, especially dietary requirements, is limited. Knowledge of optimal dietary protein level of this species is important for the development of a suitable feed for sustainable culture. A 10-week experiment was conducted to determine the optimal protein requirement of *C. nigrodigitatus* (initial weight 16.30 ± 0.07 g, mean ± SE) in twelve 60-L indoor flow through rectangular glass tanks provided with aerated underground water. Four isoenergetic diets were formulated to contain varying crude protein (CP) levels: 32.1%, 34.6%, 42.8%, and 47.1% using fish meal/soybean meal as protein sources in a ratio of 2:1. Each diet was assigned to triplicate groups of 12 fish in a completely randomized design. Twelve uniform-sized fish were stocked in each tank. A digestibility trial was conducted with all the diets. Fish was fed to apparent satiation with experimental diets twice a day. Results after ten weeks of feeding showed an increase in weight gain (WG) and specific growth rate with increasing levels of dietary protein up to 42.8% (P <0.05) but a decline at 47.1% CP. Protein efficiency ratio followed similar trend but there were no significant differences between the treatments. Feed conversion ratio (FCR) reduced as dietary protein level increased, with the minimum FCR in the 42.8% protein diet, although this was not significantly different from the 34.6% and 47.1% protein diets. The results of the present study indicate that the maximum growth of juvenile *C. nigrodigitatus* was achieved at about 42.8% dietary protein. Analysis of dose (protein level)-response (WG) with third-order polynomial regression suggested that the optimal dietary protein requirement for the juvenile of *C. nigrodigitatus* was 42.9%.

INTRODUCTION

Members of the genus *Chrysichthys* are widely distributed in the freshwater systems of Tropical Africa and support thriving commercial fisheries of many West African waters, Ghana being no exception. In Ghana Claroteid catfish forms about 40% of the catch from the Volta Lake alone. *Chrysichthys nigrodigitatus* (Lacepede, 1803) a common silver coloured African catfish of the family Claroteidae has been demonstrated to be omnivorous having a wide variety of food preferences (Offem *et al*, 2008). According to Adewolu and Benfey (2009), *C. nigrodigitatus* can be cultured in both fresh and brackish waters. Considerations for the culture of the fish have resulted in several biological studies on the species. *C. nigrodigitatus* is a relatively new species in aquaculture and the knowledge of its ecology and biology, especially its dietary requirements is still limited. *C. nigrodigitatus* is similar to *Chrysichthys maurus*. In a preliminary survey of indigenous species being tried for aquaculture in Ghana, it was thought that *C. maurus* was common on fish farms but these were later determined to be C. *nigrodigitatus* based on further information obtained about the natural distribution of the two species. The distribution of *C. maurus* in Ghana is limited to the southwestern border of the country as the species is more common in neighboring Ivory Coast. *C. nigrodigitatus*, on the other hand, is found in all the major river basins of

Ghana, making it naturally adaptable to captivity in pond systems in the country. A consultation with farmers revealed that *C. nigrodigitatus* are not only common in farmers' ponds but also they grow faster and bigger than *C. maurus.*

Although some farmers in Ghana keep *C. nigrodigitatus* in their ponds, they have little or no knowledge of the nutrient requirements and general management of the species. Few studies have been conducted on the nutrition and the recent one was by Adewolu and Benfey (2009). In determining the minimum nutrient requirements of a cultured species, protein is usually given first consideration because of its high cost and essential role for growth, tissue maintenance and reproduction. Therefore, investigating the protein requirement entails determining the minimum amount required to produce maximum growth and not to be used for energy needs. The objectives of the present study was to evaluate the effects of dietary protein levels in diets on growth performance, feed utilization, body composition and apparent digestibility coefficient (ADC) so as to determine the optimum dietary protein requirement for juvenile *C. nigrodigitatus.*

Literature review of habitat, food habits, life-history and distribution

C. nigrodigitatus is abundant and widely distributed. It is found in all the three river basins in Ghana namely; Volta (Black and White Volta Rivers, the Oti River and the Lower Volta, including Lake Volta), South Western (Bia, Tano, Ankobra and Pra Rivers) and Coastal Basins (Ochi-Amissah, Ochi-Nakwa, Ayensu, Densu and Tordzie/Aka Rivers) but *C. maurus* is restricted to the South Western basin (Dankwa et al., 1999). It is a bottom-dwelling freshwater fish widely distributed in the freshwater systems of Tropical Africa including Ghana (Nwadiaro and Okorie, 1985; Dankwa et al., 1999). *C. nigrodigitatus* is found in all the three river basins in Ghana namely; Volta, South Western and Coastal Basins (Dankwa *et al.*, 1999), consisting of numerous rivers and streams and the Volta Lake and covering about 70% of the country. *C. nigrodigitatus* is largely omnivorous (with strong tendency towards predation), with large proportions of animal component and detritus in their diet and they are adequately adapted for such habits (Offem *et al*, 2008). Juvenile members of the species have been found to be plankton feeders feeding mainly on zooplankton including copepods (Ogwumba, 1988). *C. nigrodigitatus* is known to breed seasonally and usually spawn during the rainy and flooding season. The reproductive cycle of *C. nigrodigitatus* has been reported to be around September and November, with vitellogenesis duration of 4 months (Nuñez *et al*, 1995). However, Offem *et al* (2008) indicated that *C. nigrodigitatus* has a long spawning period, extending from April to August. The completely different records on spawning season suggests that *C. nigrodigitatus* is a serial spawner with possibly two peaks in reproduction coinciding with the major rainy seasons in the humid tropics of West Africa. The species spawns in crevices (speleophil) like many catfishes, and we have seen in our experimental ponds and on some farms that under the right conditions the female enters hollow structures such as wood or bamboo to deposit its eggs.

MATERIALS AND METHODS

Experimental system and fish handling

This study was conducted at the Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Fish were reared in glass tanks (60 L) in a flow-through system, which was supplied with water at a flow rate of 0.5 L min^{-1}. Water quality parameters measured weekly during the experiment were as follows (mean ± SD): temperature, 26.76 ± 0.02^{o}C; pH, 7.22 - 6.71; dissolved oxygen, 4.04 ± 0.08 mg L^{-1}; salinity, 0.09 ± 0.00 and ammonia, 0.01 ± 0.01 mg L^{-1}. *C. nigrodigitatus* obtained from the Volta River near Akuse, Ghana were kept in hapas and fed commercial feed for three weeks before the experiment. One week before the start of the experiment, fish were transferred to the experimental tanks for acclimation. Fish of mean initial weight 16.30 ± 0.07 g were stocked randomly 12 fingerlings per tank in triplicates per treatment.

Diet formulation and preparation

All ingredients used in this study were obtained from commercial sources in Ghana. Four isoenergetic diets were formulated to contain varying crude protein (CP) levels ranging from 30%, 35%, 40%, and 45% using fish meal and soybean meal as protein sources in a ratio of 2:1 (Table 1). Fish were hand-fed twice a day (09:00, 16:00) to satiation. Feeding rates were adjusted every week and the growth experiment lasted ten weeks. At the end of the growth trial feces were collected for digestibility study by siphoning into centrifuge bottles and immediately centrifuged using Universal 16A, Centrifuge at 3,000 rpm for 10 min and the supernatant discarded. Feces were then oven dried at 60^{o}C for 24h, ground into a fine powder and stored in a desiccator for chemical analysis.

Table 1. Composition of diets fed to *Chrysichthys nigrodigitatus* with varying protein levels.

	Protein levels (g/100 g as-fed)			
Ingredients	30	35	40	45
Fish meal	23.7	29.8	35.0	40.7
Soy bean Meal	18.8	22.2	27.5	32.2
Rice Bran	30.5	23.0	15.0	8.0
Wheat Bran	15.5	13.5	11.0	7.6
Salt	1.0	1.0	1.0	1.0
Palm oil	2.0	2.0	2.0	2.0
Di Calcium Phosphate	2.0	2.0	2.0	2.0
Cassava Flour (Binder)	2.0	2.0	2.0	2.0
Vitamin & mineral Premix	4.0	4.0	4.0	4.0
Chromic Oxide	0.5	0.5	0.5	0.5
Proximate Composition (%)				
Dry matter	95.2	95.5	96.7	96.7
Crude protein	32.1	34.6	42.8	47.1
Crude lipid	9.1	8.8	10.7	14.5
Crude fibre	7.3	4.9	4.3	
Ash	15.9	15.9	15.9	18.2
Gross energy, (kJ g^{-1})	17.3	17.8	18.9	19.6

Biochemical, biological and statistical analyses

Ingredients, diets, carcass and feces were analyzed in triplicates for proximate composition according to standard methods (AOAC, 1990) and chromic oxide of diets and feces analyzed using the method by Furukawa and Tsukahara (1966). Performance in growth and feed utilization were determined in terms of weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) as follows: WG (%) = final body weight – initial body weight/ initial body weight x 100; SGR (% day^{-1}) = 100 × [ln(final body weight) - ln(initial body weight)]/no. of days; FI (g) = Total feed intake per fish/no. of days; FCR = feed intake/live weight gain; PER = live weight gain/crude protein intake. The apparent digestibility coefficients (ADC) for the nutrients of the diets were calculated as follows: ADC (%)=100×[1-(%nutrient in feces/%nutrient in feed)×(%marker in feed/marker in feces)]. Whole body composition was determined where whole body samples were analyzed for moisture, crude protein, crude lipid and ash and results expressed as percentage of live weight. All data were subjected to ANOVA using SPSS 16.0. Differences between the means were tested by Tukey's test ($\alpha = 0.05$). The optimum dietary protein requirement for juvenile *C. nigrodigitatus* based on percentage weight gain (WG) was estimated by third-order polynomial regression analysis ($Y = a + bX + cX^2 + dX^3$) (Neter *et al.,* 1996).

The present study indicated that dietary protein level had an effect on growth performance, and the optimum protein level in diets for *C nigrodigitatus* juvenile, defined by the percentage WG, was 42.9% when fish meal and soybean meal were used as the main protein source and the dietary energy value was 18.9 kJ/g. This value was similar to those used in omnivorous fish diet, for example, bagrid catfish, *Mystus nemurus* (44%; Ng *et al.* 2001); silver perch, *Bidyanus bidyanus* (42.2%; Yang et al. 2002); black sea bream, *Sparus macrocephalus* (41.4%; Zhang *et al.* 2010) but higher than those reported for other omnivorous species, such as, channel catfish *Ictalurus punctatus* (28-32%; Robinson *et al*. 2000) and *Heterotis niloticus* (30.6-31.1%; Monentcham, 2009). Adewolu and Benfey (2009) also studied protein requirements for juvenile *C. nigrodigitatus* and reported at least 35% dietary protein and stated that optimum protein requirement was not established for the species because no growth plateau was reached.

RESULTS AND DISCUSSION

Growth performance of *C. nigrodigitatus* fed the diets containing varying dietary protein levels for 10 weeks is presented in Table 2. There was no significant difference in the initial body weight of *C. nigrodigitatus*; however, after the 10-wk feeding trial, the fish grew and weight gain (WG) ranged between 21.3 and 44.9%, and the fish fed 42.8% dietary protein showed the highest WG value (44.9%) ($P < 0.05$) than other treatments except for fish fed 47.1% protein diet. SGR increased significantly with increasing dietary protein levels up to 42.8% protein diet (0.53% day^{-1}) and decreased slightly at 47.1% protein (0.40% day^{-1}).

Table 2. Growth responses and feed utilization of juvenile *Chrysichthys nigrodigitatus* fed varying levels of protein for 70 days.

	Crude protein levels in the diet (%)			
Parameter	32.1	34.6	42.8	47.1
Initial weight (g)	16.42 ± 0.05	16.34 ± 0.07	16.10 ± 0.27	16.35 ± 0.10
Final weight (g)	19.91 ± 0.31^{b}	20.40 ± 0.48^{b}	23.32 ± 0.33^{a}	21.67 ± 0.72^{ab}
Weight gain (g)	3.49 ± 0.36^{b}	4.06 ± 0.42^{b}	7.22 ± 0.31^{a}	5.31 ± 0.64^{ab}
Weight gain (%)	21.25 ± 2.29^{a}	24.85 ± 2.46^{a}	44.88 ± 2.29^{b}	32.46 ± 3.78^{ab}
Specific growth rate (%.day^{-1})	0.28 ± 0.03^{b}	0.32 ± 0.03^{b}	0.53 ± 0.03^{a}	0.40 ± 0.04^{ab}
Feed intake (g)	22.11 ± 0.12	22.18 ± 1.30	23.83 ± 0.24	21.27 ± 0.67
Feed conversion ratio	6.48 ± 0.67^{b}	5.63 ± 0.83^{ab}	3.32 ± 0.18^{a}	4.13 ± 0.56^{ab}
Protein intake (g)	7.09	7.12	7.64	6.82
Protein efficiency ratio	0.49 ± 0.05	0.54 ± 0.09	0.71 ± 0.04	0.53 ± 0.07
Survival (%)	80.55 ± 2.78	83.33 ± 9.62	77.78 ± 2.78	88.89 ± 5.56

Values are presented as means ± SE (n = 3) and values within the same row with different letters are significantly different ($P < 0.05$).

Growth, as expressed by percentage weight gain and specific growth rate, increased with increasing dietary protein level within the range of 32.1%–42.8% crude protein. Increases in dietary protein have often been associated with higher growth rates in many species as this component provides the essential amino acid building blocks for protein synthesis (McGoogan *et al.* 1999). Third-order polynomial regression analysis based on WG ($y = 38.47 + 2.884(x - 39.15) - 0.182(x - 39.15)^2 - 0.035(x - 39.15)^3$; adjusted $R^2 = 0.783$; x = dietary protein levels (%), y = WG (%)) showed that the optimum dietary protein level was 42.9% (Fig 1).

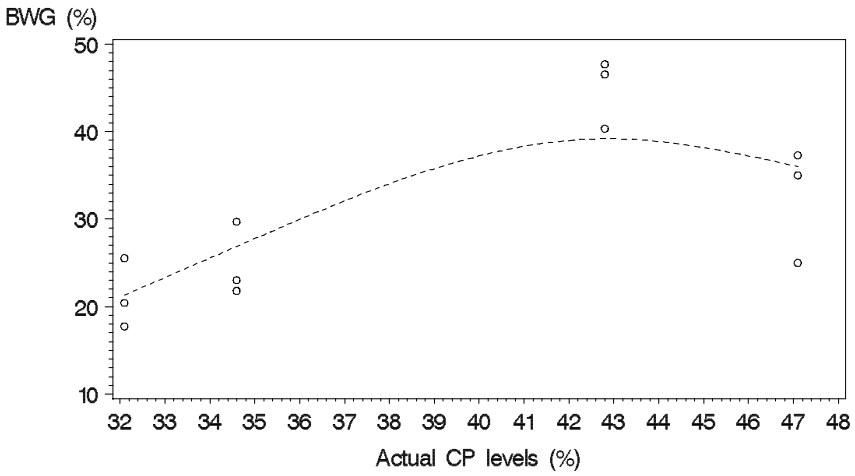


Fig 1 The relationship between weight gain (%, *y*) and dietary protein levels (%, *x*) in juvenile *C. nigrodigitatus.*

The present study indicated that dietary protein level had an effect on growth performance, and the optimum protein level in diets for *C nigrodigitatus* juvenile, defined by the percentage WG, was 42.9% when fish meal and soybean meal were used as the main protein source and the dietary energy value was 18.9 kJ/g. This value was similar to those used in omnivorous fish diet, for example, bagrid catfish, *Mystus nemurus* (44%; Ng *et al.* 2001); silver perch, *Bidyanus bidyanus* (42.2%; Yang et al. 2002); black sea bream, *Sparus macrocephalus* (41.4%; Zhang *et al.* 2010) but higher than those reported for other omnivorous species, such as, channel catfish *Ictalurus punctatus* (28-32%; Robinson *et al*. 2000) and *Heterotis niloticus* (30.6-31.1%; Monentcham, 2009). Adewolu and Benfey (2009) also studied protein requirements for juvenile *C. nigrodigitatus* and reported at least 35% dietary protein and stated that optimum protein requirement was not established for the species because no growth plateau was reached. Protein requirements between fish species is complicated by differences in species, size and age of fish, diet formulation, stocking density, protein quality, hygiene and experimental conditions between studies (NRC 1993).

Feed utilization by *C. nigrodigitatus* improved as dietary protein level increased. Feed intake (FI) increased slightly with the increase in dietary protein up to 42.8% (23.8 g) but reduced a bit at 47.1% (21.3 g) however, there was no significant differences between treatments. FCR data showed approximately the opposite pattern as FI with diet containing 42.8% protein being better utilized (3.3) than the others, although it was not significantly different from fish fed 47.1% protein diet. The observation that (FCR) decreased with increasing dietary protein levels has also been reported for many other species, irrespective of culture conditions, including Mozambique tilapia (Jauncey 1982), African catfish (Degani et al. 1989) and Bagrid catfish (Adewolu and Benfey, 2009). The high FCR values (3.3–6.5) observed in this study may be a result of the use of locally available cheap ingredients for practical diet formulation; high FCR values have been reported for a number of fish species fed on practical diets using locally available feed ingredients (Khan *et al.* 1993). This also probably led to low growth performance, although water supply problems were encountered during the experiment, which could have been one of the reasons. In a majority of similar studies, researchers employed various purified and semi purified diets with high quality protein sources such as casein, gelatin or synthetic amino acids that gave good growth and feed utilization leading to more precise values.

Feed utilization by *C. nigrodigitatus* improved as dietary protein level increased. Feed intake (FI) increased slightly with the increase in dietary protein up to 42.8% (23.8 g) but reduced a bit at 47.1% (21.3 g) however, there was no significant differences between treatments. FCR data showed approximately the opposite pattern as FI with diet containing 42.8% protein being better utilized (3.3) than the others, although it was not significantly different from fish fed 47.1% protein diet. The observation that (FCR) decreased with increasing dietary protein levels has also been reported for many other species, irrespective of culture conditions, including Mozambique tilapia (Jauncey 1982), African catfish (Degani et al. 1989) and Bagrid catfish (Adewolu and Benfey, 2009). The high FCR values (3.3–6.5) observed in this study may be a result of the use of locally available cheap ingredients for practical diet formulation; high FCR values have been reported for a number of fish species fed on practical diets using locally available feed ingredients (Khan *et al.* 1993). This also probably led to low growth performance, although water supply problems were encountered during the experiment, which could have been one of the reasons. In a majority of similar studies, researchers employed various purified and semi purified diets with high quality protein sources such as casein, gelatin or synthetic amino acids that gave good growth and feed utilization leading to more precise values.

ADCs of protein, organic matter and lipid increased as protein content of the diets increased (Table 3) but the increase was not significant for ADCs of protein and organic matter. However, ADC of lipid showed a significant increase as dietary protein content increased with 47.1% protein diet recording the highest (91.33%).

Table 3. Apparent digestibility coefficients (%) of main nutrients in diets for juvenile *Chrysichthys nigrodigitatus* fed varying levels of protein for 70 days.

	Crude protein levels in the diet (%)			
	32.1	34.6	42.8	47.1
Crude protein	70.59 ± 4.78	71.60 ± 4.67	72.57 ± 1.08	71.75 ± 1.46
Crude lipid	83.41 ± 2.67^{b}	88.83 ± 0.30^{ab}	88.91 ± 1.96^{ab}	91.33 ± 0.96^{a}
Dry matter	49.29 ± 2.83	53.05 ± 2.59	55.78 ± 2.43	56.36 ± 0.56

Values are presented as means ± SE (n = 3) and values within the same row with different letters are significantly different (P< 0.05).

Whole body composition of *Chrysichthys nigrodigitatus* is presented in Table 4. Percentage moisture, ash content, whole-body protein were not significantly affected by dietary protein level (P>0.05). The whole-body protein was positively correlated with dietary protein level. Whole-body lipid and ash also followed a similar trend up to 42.8% dietary protein and decline slightly at 47.1%. Percentage moisture was negatively correlated with dietary protein level. Increasing dietary protein level increased lipid content in body composition significantly (P <0.05). Similar results were observed in the carcass composition of Nile tilapia (El-Saidy & Gaber 2005) and Silver perch (Yang *et al*. 2002).

Table 4. Whole body composition (% wet weight) and energy of juvenile *Chrysichthys nigrodigitatus* fed varying levels of protein for 70 days.

Components	Crude protein levels in the diet (%)			
	32.1	34.6	42.8	47.1
Moisture content	75.66 ± 0.61	75.56 ± 1.67	73.69 ± 0.23	73.26 ± 1.18
Crude protein	15.77 ± 0.83	16.39 ± 1.22	19.12 ± 0.30	19.37 ± 0.94
Crude lipid	2.83 ± 0.46^{b}	4.05 ± 0.54^{ab}	5.00 ± 0.31^{a}	4.23 ± 0.33^{ab}
Ash	3.58 ± 0.15	4.34 ± 0.65	4.96 ± 0.75	4.65 ± 0.22

Values are means ± SE (n = 3) and values within the same row with different letters are significantly different (P< 0.05).

CONCLUSION

The results of the present study indicate that the maximum growth of juvenile *C. nigrodigitatus* was achieved at about 42.8% dietary protein when fish and soybean meals were used as the major sources of protein. Using a polynomial regression analysis of WG, the dietary protein requirement for the juvenile of *C. nigrodigitatus* was estimated to be 42.9%.

Ecology And Dietary Protein Requirement Of Juvenile African Bony-Tongue, *Heterotis Niloticus*

ABSTRACT

The study evaluated the effect of four isoenergetic diets with varying crude protein (CP) levels of 26.2%, 32.1%, 34.6% and 42.8% on growth, feed utilization and whole body proximate composition of *Heterotis niloticus* juveniles. *H. niloticus* juveniles (initial weight 32.65±0.03g) were stocked in rearing hapas ($2x1m^2$) at 5 fish per hapa. Each diet was assigned to triplicate groups of fish in a completely randomized design and the experiment lasted for ten weeks. An increasing growth trend and better feed utilization was observed as dietary protein levels increased from 26.2% to 42.8%. Fish fed 42.8% protein diet had the best growth performance and nutrient utilization, with a mean weight gain of 202.30±19.6%, feed conversion ratio of 1.20±0.15 and protein efficiency ratio of 1.66±0.2, however this was not significantly different from values of fish fed 32.1% and 34.6% dietary protein. Significantly lower values were recorded for fish fed 26.2% dietary protein. Whole body nutrient composition was not affected by the diets. The results of this study suggest that *H. niloticus* juveniles would grow best when fed diets containing at least 32.1% protein.

INTRODUCTION

Among the most highly valued species in West African inland fisheries is the African bony tongue, *Heterotis niloticus* (Cuvier, 1829), a species widely distributed in tropical rivers, freshwater lakes of Western and Central Africa (Moreau 1982). The African bony tongue is exploited by fisheries in Southern Benin, the middle Niger River Delta and other regions of West Africa (Adite *et al*., 2005). The species is classified within the opportunistic omnivorous fish category and consume a variety of food resources, ranging from aquatic invertebrates to small seeds, including small benthic organisms, fishes, shrimps, plant remains and terrestrial insects. In captivity, remarkable growth performances have been reported, individual mean body mass reaching up to 3 to 4 kg in 12 months. However, its use for profitable fish-farming in Africa relies on the knowledge of ecological, behavioral and nutritional factors which condition its reproduction, the resolution of massive mortality during early rearing, the estimate of its nutritional needs at various ontogenetic stages and the identification of an efficient breeding. The prospect for Heterotis contribution to the rise of African aquaculture depends on the solutions which will be found to the mentioned crucial problems (Monentcham, 2009). In spite of the great evolutionary and fishery significance, the bony tongue fishes of the family Osteoglossidae generally have not received extensive study (Adite *et al*., 2005). Considering the bony tongue's great ecological and economic importance in Ghana, more information is needed on natural feeding habits and nutritional requirements of the species in order to inform both fisheries management and development of aquaculture technology. This study, therefore was undertaking to evaluate the growth performance, feed utilization and whole body composition of *H. niloticus* fed on varying dietary protein levels.

Literature review of habitat, food habits, life-history and distribution

H. niloticus is a pelagic freshwater fish usually found in waterbodies with aquatic vegetation (e.g., Swamps) particularly during spawning seasons (Adite *et al.,* 2005). The African bony tongue has been characterized as microphagous on phytoplankton (Lowe-McConnell, 1975; Holden and Reed, 1991) and feeding on variable amounts of plant material, including seeds, and benthic water column invertebrates. *H. niloticus* spawn in nests, which are circular clearings within dense stands of rooted and submerged or emergent aquatic macrophytes in shallow water (Padi, 2006; Adite *et al.,* 2005). Spawning occurs during the rainy seasons in Ghana. *H. niloticus* occurs in large rivers of West Africa. In Ghana it occurs mainly in the Volta basin (Dankwa *et al*., 1999).

MATERIALS AND METHODS

Experimental system and diet preparation

This study was carried out at the premises of the Data Stream Hatchery at Old Akrade in the Eastern Region of Ghana. Twelve hapas ($2x1m^2$) mounted in concrete tanks ($5x20\ m^2$) were used for the feed trial. Before mounting the hapas, the concrete tank was cleaned with calcium carbonate to kill bacteria and other micro-organisms and left to dry for about a week and then filled with water from the Volta River. The concrete tank was fitted with an air blower for water aeration and ultra violet clarifier for water disinfection. All ingredients used for diet preparation in this study were obtained from commercial sources in Ghana. Four isoenergetic diets were formulated to contain varying crude protein (CP) levels of 25%, 30%, 35%, and 40%, using fish meal and soybean meal as protein sources in a ratio of 2:1 (Table 1).

Table 2. Composition of diets fed to *Heterotis niloticus* with varying protein levels.

Ingredients	Protein levels (g/100 g as-fed)			
	25	30	35	40
Fish meal	18.0	23.7	29.8	35.0
Soy bean Meal	13.9	18.8	22.2	27.5
Rice Bran	38.0	30.5	23.0	15.0
Wheat Bran	18.6	15.5	13.5	11.0
Salt	1.0	1.0	1.0	1.0
Palm oil	2.0	2.0	2.0	2.0
Di Calcium Phosphate	2.0	2.0	2.0	2.0
Cassava Flour (Binder)	2.0	2.0	2.0	2.0
Vitamin & mineral Premix	4.0	4.0	4.0	4.0
Chromic Oxide	0.5	0.5	0.5	0.5
Proximate composition				
Dry matter	97.0	95.2	95.5	96.7
Crude protein	26.2	32.1	34.6	42.8
Crude lipid	9.1	9.1	8.8	10.7
Crude fibre	8.56	7.3	4.9	4.3
Ash	13.6	15.9	15.9	15.9
Gross energy, ($kJ\ g^{-1}$)	16.9	17.4	17.9	18.4

Experimental fish, acclimation, stocking and feeding

A total of sixty Fingerlings of *H. niloticus (*initial weight of 32.7g*)* obtained from Zewu Farms, Akuse were used for the study. The fish were randomly stocked 5 fish per hapa in triplicates per treatment. The fish were allowed to acclimatize for two weeks prior to the start of the feeding trial. During this period, the fish were fed on (crumbles) imported feed with 45% crude protein (Raanan feed from Israel). The fish

were hand-fed to satiation twice daily at 0700h and 1600h. Feeding rates were adjusted every week and the growth experiment lasted ten weeks.

Biochemical, biological and statistical analyses

Ingredients, diets, carcass and feces were analyzed in triplicates for proximate composition according to standard methods (AOAC, 1990). Performance in growth and feed utilization were determined in terms of weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) as follows: WG (%) = final body weight – initial body weight/ initial body weight x 100; SGR (% day^{-1}) = 100 × [ln(final body weight) - ln(initial body weight)]/no. of days; FI (g) = Total feed intake per fish/no. of days; FCR = feed intake/live weight gain; PER = live weight gain/crude protein intake. Whole body composition was determined where whole body samples were analysed for moisture, crude protein, crude lipid and ash and results expressed as percentage of live weight. All data were subjected to ANOVA using SPSS 16.0. Differences between the means were tested by Tukey's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

In this study, varying inclusion levels of dietary protein had effect on the growth performance (FBW, WG and SGR) and feed utilization (FI, FCR and PER) of *Heterotis niloticus* ($P< 0.05$; Table 2). FBW, WG and SGR increased with increasing dietary protein levels with the maximum values in the 42.8% protein diet, although this was not significantly different from the 32.1% and 34.6% protein diets. FI and PER followed a similar trend but there was no significant differences between the diets. The reverse trend was true for FCR with the least value (1.2) recorded for fish fed 42.8% protein diet, but did not differ significantly from the 32.1% and 34.6% protein diets. The survival rate generally showed a decreasing trend with increasing dietary protein level but was not significantly different between the diets.

Table 2. Growth and feed utilization of *H. niloticus* fed at varying inclusion levels of dietary protein for 70 days.

Parameter	Crude protein levels in the diet (%)			
	26.2	32.1	34.6	42.8
IBW	32.30±0.40	32.73±0.03	32.80±0.06	32.77±0.03
FBW	65.56±4.57^{a}	76.12±9.07^{ab}	90.40±7.19^{ab}	99.07±6.50^{b}
WG	102.80±12.42^{a}	132.60±27.77^{ab}	175.6±21.85^{ab}	202.30±19.56^{b}
FCR	1.85±0.17^{a}	1.77±0.13^{ab}	1.69±0.07^{ab}	1.20±0.15^{b}
SGR	0.90±0.08^{a}	1.06±0.15^{ab}	1.29±0.11^{ab}	1.41±0.08^{b}
SUR	93.33±6.67	91.67±8.33	73.33±13.33	71.67±17.40
PER	1.27±0.16	1.44±0.30	1.65±0.21	1.66±0.16
FI	60.49±3.45	74.33±10.14	76.53±8.69	77.45±3.0630
K	2.46±0.16	2.48±0.29	2.19±0.14	2.06±0.24

IBW (g)=Initial body weight, FBW (g)=Final body weight, WG (%)=Weight gain, FCR=Feed conversion ratio, SGR (%day^{-1})= Specific growth rate, SUR (%)= Survival, PER=Protein efficiency ratio, FI (g)=Feed intake, K= Condition factor. Values are presented as means ± SE (n = 3) and values within the same row with different letters are significantly different ($P< 0.05$)

These results are in agreement with (Al-Hafedh *et al*., 1999) who found that better growth of Nile tilapia was obtained at high dietary protein levels 40-45 % rather than 25-35 % protein in the absence of live algae and (Tacon, 1987) who found that dietary protein level varied from 42% for fry and 35% for growing adult. It also compares with Monentcham, (2009) who concluded that maximum growth of

Heterotis fingerlings was achieved at about 34.5% protein, however using the broken line model the dietary protein requirement was estimated to be 31.0%.

The decrease in FCR with increase in dietary protein in this study, suggests a corresponding increase in feed conversion efficiency of the fish in accordance with the fact that protein is the major dietary nutrient affecting performance of fish (Lovell, 1989) since it provides the essential and nonessential amino acids which are necessary for muscle formation and enzymatic function and in part provides energy for maintenance.

The condition factor (K) was not affected by the varying levels of protein inclusion in the diets. The K values range from 2.06 to 2.46 (Table 2). These results are in close agreement with those of (Osman, 1991) who reported that K values in general, for fish ranged between 2.20 and 2.33 and also agreed with those of (Ahmad *et al.*, 2004).

There were no significant differences ($P<0.05$) in the whole body composition of the fish (Table 4.5) among the diets in relation to protein, moisture, crude lipid, crude fibre, ash and nitrogen free extracts at the end of the feeding trial. The whole body protein, lipid, moisture and ash contents were not were not significantly affected by dietary protein levels (Table 3). Similar results were observed in *H. niloticus* (Monentcham, 2009) and Nile tilapia (El-Saidy & Gaber 2005).

Table 3. Whole body compositions (% wet weight) of *H. niloticus* fed varying inclusion levels of protein for 70 days.

Parameter	Initial value	Crude protein levels in the diet (%)			
		26.2	32.1	34.6	42.8
Moisture content	74.25 ± 0.26	76.02±0.71	75.22±0.72	76.35±0.49	75.03±0.95
Crude protein	18.66 ±0.21	17.77 ±0.42	18.40 ±0.56	18.43±0.38	18.54±0.71
Crude lipid	1.66± 0.03	1.65±0.12	1.57±0.05	1.69±0.03	1.71±0.03
Ash	1.59±0.04	1.51±0.14	1.51±0.04	1.44±0.04	1.56 ±0.10

Values are means ± SE (n = 3) and values within the same row with different letters are significantly different ($P< 0.05$).

CONCLUSION

The results of this study shows that the maximum growth and feed utilisation of *H. niloticus* fingerlings were achieved when dietary protein was about 42.8% using practical diets with fish and soybean meals as the major sources of protein. In conclusion, the use of a practical diet containing at least 32.1% protein would be appropriate for growth and nutrient utilization of juvenile *H. niloticus* under the conditions of this study.

Ecology And A Survey Of Market Potential And Distribution Trends Of Snakehead, *Parachanna Obscura*, In Four Regions In Ghana

While the original objective of this project included experiments to determine the dietary protein requirements of juveniles of the three species, initial laboratory trials with *Parachanna obscura* failed because the individuals kept in tanks refused pelleted feed, although they would readily take

live feed like tilapia. Experimental study of *Parachanna obscura* dietary protein requirements was therefore not feasible. A more in-depth study of the distribution and natural food and habitat requirement and acceptability as a potential aquaculture species in Ghana completed in lieu of the experimental diet studies.

Literature review of habitat, food habits, life-history and distribution

Snakeheads are voracious predators (Fagbenro, 2002) and have been observed to exhibit ontogenetic changes in their diets. Juveniles of *P. obscura* prey on insects' larvae, nematodes and fish fry whilst adults prey on larger fish (USEPA, 2002, Ajah et al., 2006). Juveniles have been reported as benthic feeders (Ajah et al., 2006). When starved, some snakeheads have a tendency to be cannibalistic on their young (USEPA 2002). Snakeheads are likely to be encountered in flood plains, both in the open water and the swamps (Oti, 2003; Brummet and Teugels, 2002). In rivers and streams, they have been reported to occur in vegetated areas (USEPA, 2002). All the species in the family Channidae are air-breathing, therefore, they can survive hypoxic conditions as early as late juvenile stages (USEPA, 2002; UGSS, 2010a). Optimum pH ranges are reported to differ among species in the family (UGSS 2010a). Information on spawning activities in African snakehead is lacking. However, spawning has been observed in summer (June to August) in many of the species in the family Channidae (USEPA, 2002). Fecundity in *P. obscura* in a monoculture farm in Nigeria (Victor and Akpocha, 1992) was reported to be variable and highest in October and November with 35- 4,010 eggs. Some species are also capable of spawning up to five times in a year (USGS, 2010a). For mouth brooding species, fecundity is very low ranging from about 20 eggs at first maturity and increasing to about 200 as the fish grows (USGS, 2010a). Prior to spawning, adults build circular nests in water columns in vegetated areas where spawning occurs (Gascho Landis and Lapointe, 2010). However, presence of vegetation may not always be a prerequisite for spawning because some species in the genus *Channa* have been reported to spawn in the absence of vegetation (USGS, 2010a). Higher temperatures appear to speed up hatching rates of eggs among species; eggs were observed to hatch in 45-120 hours at 16°C-26°C and 28-31 hours at 28°C-33°C (USGS, 2010a). Fry begin to feed on zooplankton when yolk sacs are depleted (USGS, 2010a). Snakeheads are very aggressive in protection of their young. Parental care in the family has been observed to differ from species to species but all species exhibit some parental care (USEPA, 2002).

Description of the study areas

The study was conducted in the Ashanti, Brong Ahafo, Eastern and Western Regions of Ghana. The selection of these regions was as a result of the apparent availability of snakehead (*Parachanna obscura*) from rivers in the regions. The specific towns surveyed include Barekese (Ashanti), Offinso (Offinso), Ekye-Amanfrom (Eastern), Sefwi- Wiawso (Western), Techiman (Brong Ahafo) and Yeji (Brong Ahafo). These towns both had rivers or reservoirs within which thrived active fishing. In addition, the towns had markets where a market survey could be conducted.

METHODS

Sample selection and survey data Collection

The study was conducted with fishermen/women and fish traders in the four regions selected. Due to lack of a sampling frame for both fisher folk and fish traders, we resulted to total population sampling when the numbers were small (40 or below) and random sampling when the numbers were large (above 200). Fortunately, all fisher folk encountered were willing to be interviewed but some fish traders (about 10) were not available by their goods or unwilling to be interviewed. Apart from Yeji and Sefwi-wiaso where the fish traders were randomly surveyed, we employed total sampling in all the markets. A total of 177 respondents were surveyed comprising 51 fisher folk (including one woman) and 126 fish traders (including one man).

The survey was conducted between July and August 2011. The fisher folk were interviewed at the river side, their homes or market centers. Most of the fish traders were interviewed at the market centers with a few in the Eastern Region interviewed at their homes. We administered all 177 questionnaires through personal interviews. To avoid confusion about the species being studied, we presented a photograph and a sample of the species to all respondents.

Questionnaire design

We employed both close-ended and open-questions in the survey and used multiple measures to assess changes in snakehead distribution in the four regions. These include frequency of encounter with snakehead, towns where snakehead are caught or purchased and the rivers from which they are caught. To assess relative abundance of snakehead, fisher folk were asked to indicate their current estimated catches. In addition, they had to indicate if they had observed any changes in catches and to provide information about the changes. The snakehead market potential was assessed through asking respondents to compare the price of snakehead to other commonly sold species. We also assessed market potential by asking respondents to indicate whether or not they are meeting the demand for snakehead.

RESULTS AND DISCUSSION

Distribution, abundance, and market potential of snakehead in Ghana

Data analysis for the survey is ongoing but preliminary findings suggest that snakeheads thrive in the major watersheds in Ashanti, Brong Ahafo, Eastern, and Western Regions. The rivers mentioned by fishermen as their sources of snakehead include Afram, White Volta, Subin, Offin, and Tano. This was corroborated by the market women who also provided town names such as Yeji, Bupe, Afram plains, Kwahu and Kumasi as their sources of fish for trade. When asked how frequently they encountered snakehead, majority of the fisher folk and fish traders said “once in a while”. A few fishermen said they encountered them daily if they set traps for them.

Species abundance appears to be driven by type of gear used and the seasonal patterns. However, seasonal patterns seem the common determinant of abundance for all four regions. The fisher folk best described the species as one that fluctuates in abundance yearly. This fluctuation occurs because of the two major seasons in Ghana. When the rivers overflow their banks between April to July, flood water which collects in pools in vegetated areas provide spawning habitats for snakehead. Subsequently, the fishermen target these areas and encircle them. Even with their traditional gill nets, large numbers of snakehead are caught. While they could only capture two to three snakehead in a typical week, they obtain as many as 1000 between August and December when the water recedes. For the fishermen who target snakehead every day of the year, relying on the same gears used for the open water species is not an option. They used baited hooks, since the species is carnivorous, to ensure catches about three snakeheads daily.

Comparing snakehead to commonly caught species such as tilapias and catfishes, the snakehead market is not well established. Snakeheads are typically processed before they are sold on the market. The most common processing method is smoking. However, on some occasions, they are salted. Only one trader indicated she sometimes sold them fresh. With the exception of Barekese (Ashanti Region) and Afram plains (Eastern Region) where we encountered samples from fishermen and on the open market, all other market had no snakehead. Most of the respondents who were familiar with the species suggested it was a high quality fish which they relished. To support that, they explained that since snakehead catches were low compared to Tilapia and Chrysichthys, they seldom sold snakehead. Evidently, snakeheads were rarely seen on the market. Snakeheads were largely not sold separately as other species. Due to the low catches, fisher folk and fish traders alike sold them mixed with other species such as Chrysichthys. Some traders indicated they discarded them hence it was difficult to determine its price. The fisher folk also added that their neighbors often bought snakeheads before they could be sent to the market. When

snakeheads were sold separately, it appears they sold just like Clarias with the same size or weight. However, this was not a general consensus. Depending on who bought the fish, the price was higher or lower compared to Tilapia or Clarias. With respect to demand for snakeheads, most of the respondents indicated they rarely had customers requesting for snakeheads. Nevertheless, many respondents suggested they would be able to sell more snakeheads if they obtained numbers above current levels.

Training

Besides several MS student theses supported at KNUST, this study provided two short-term trainings that were part of its original objectives. The first training, in Experimental Design and Analysis for Aquaculture Professionals, was conducted in July 2010 at the Providence Hostel in Kumasi, hosted by KNUST. The workshop, taught by Dr. Emmanuel Frimpong, was attended by 20 participants, including technicians and aquaculture scientists from KNUST, the Water Research Institute, and the Fisheries Commission and AquaFish-supported graduate students. The second training was a symposium on the culture of indigenous species, held at the Independence Hall of KNUST in July 2010. This symposium was attended by 151 participants and led by Drs, Nelson Agbo, Steve Amisah, Emmanuel Frimpong, and Ms. Gifty-Anane Taabeah.

PRELIMINARY CONCLUSION AND RECOMMENDATION

Snakeheads occur in major river basins in the Ashanti, Brong Ahafo, Eastern and Western regions of Ghana. Currently, their catches are low, resulting in fewer numbers reaching local markets. Even though the snakehead market is not developed like that of Tilapia or Clarias, the species has potential to penetrate local markets if the public is well educated about the species as an alternative food fish. In view of the current findings, a countrywide survey of the market potential of snakeheads will be a good study to pursue. This should provide a clearer picture of potential markets for the species. Commercial aquaculture appears a vital avenue for ensuring snakehead availability on the market and should be explored.

ACKNOWLEDGEMENT

Collaboration with the Fisheries Commission and Water Research Institute of Ghana ensured the integrity of data collected and the cooperation of participants. We thank all participants of the surveys for their cooperation. We are grateful to Yaw Ansah for his assistance with questionnaire administration. We are also grateful to Mark Yeboah-Agyepong, Faustina Asante and the technicians of the Department of Fisheries and Watershed Management, KNUST for their help with field and laboratory work.

LITERATURE CITED

Adewolu M. A., and T. J. Benfey. 2009. Growth, nutrient utilization and body composition of juvenile bagrid catfish, *Chrysichthys nigrodigitatus* (Actinopterygii: Siluriformes: Claroteidae), fed different dietary crude protein levels. Acta Icthyologica et Piscatoria. 39(2):95-101.

Adite, A., K. O. Winemiller, and E. D. Fiogbe´. 2005. Ontogenetic, seasonal and spatial variation in the diet of *Heterotis niloticus* (Osteoglossiformes: Osteoglossidae) in the Soˆ River and Lake Hlan, Benin. West Africa Environmental Biology of Fishes. 73:367–378.

Ajah, P. O., M. N. Georgewill, M. O. Ajah. 2006. The food and feeding habits of five freshwater and brackish-water fish species in Nigeria. African Journal of Aquatic Science. 31(2):313 – 318.

Ahmad, M. H., M. Abdel-Tawwab, and Y. A. E. Khattab. 2004. Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (*Oreochromis niloticus* L.) with different initial body weights. Proceedings 6th International Symposium on Tilapia in Aquaculture. 12-16 September 2004, Manila, Philippines. Pages 249-263.

Al Hafedh, Y. S. 1999. Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus* (L). Aquaculture Research. 30:385–393.

Association of Official Analytical Chemists. 1990. Official methods of analytical of the
Association of Official Analytical Chemists, 15th edition. AOAC, Arlington, Virginia,USA.
Brummet, R. E., and G. G. Teugels. 2002. Rainforest Rivers of Central Africa: biogeography and sustainable exploitation. Draft Paper. October, 22. 12pp.
Dankwa, H. R., Abban, E. K. and Teugels, G. G. (1999). Freshwater fishes of Ghana: Identification, distribution, ecological and economic importance. Annales Science Zoologiques, Vol. 283.
Degani, G., Y. Ben-Zvi, and D. Levanon. 1989. The effect of different protein levels and temperatures on the feed utilization, growth and body composition of *Clarias gariepinus* (Burchell 1822). Aquaculture. 76:293–301.
El-Saidy, D. M. S. D., and M. M. A. Gaber. 2005. Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. Aquaculture Research. 36:163-171.
Fagbenro O. A. 2002. Predator control of overpopulation in cultured tilapias and the alternative uses for stunted tilapias in Nigeria. Available at: http://ag.arizona.edu/azaqua/ista/ista6/ista6web/pdf/634.pdf
Furukawa, A., and H. Tsukahara. 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility in fish feed. Bulletin of the Japanese Society of Scientific Fisheries. 32:502–508.
Gascho Landis, A., and N. W. R. Lapointe. 2010. First record of a northern snakehead (*Channa argus* Cantor) nest in North America. Northeastern Naturalist 17(2):325-332.
Holden, M. and Reed, W. (1991). West African freshwater fish. Longman Group Ltd., London
Jauncey, K. 1982. The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). Aquaculture. 27:43-54.
Khan M. S., K. J. Ang, M. A. Ambak, and C.R. Saad. 1993. Optimum dietary protein requirement of a Malaysian freshwater catfish, *Mystus nemurus*. Aquaculture. 112:227–235.
Lovell, R. T. 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold (ed.). New York. 260p.
McGoogan, B. B. and D. M. Gatlin III. 1999. Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus* I. Effects of dietary protein and energy levels. Aquaculture. 178:333–348.
Monentcham, S. 2009. Alimentation et nutrition des juvéniles de *Heterotis niloticus* (Arapaimidae, Teleostei). PhD thesis. Presses Universitaires de Namur, Namur.
Moreau, J. 1982. Exposé synoptique des données biologiques sur *Heterotis niloticus* (Cuvier, 1829). FAO Synop. Pêches (131), p. 45.
Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. Applied linear statistical models, fourth edition. McGraw-Hill, New York, 1408pp.
Ng, W. K., S. C. Soon, and R. Hashim. 2001. The dietary proteinrequirement of a bagrid catfish,
Mystus nemurus (Cuvier & Valenciennes), determined using semi-purified diets of varying protein level. Aquaculture Nutrition. 7:45–51.
NRC .1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC.
Nunez Rodriguez J., Ziriga Josilé Otémé and Saurin Heni, 1995: Comparative study of vitellogenesis of two African catfish species *Chrysichthys nigrodigitatus* (Claroteidae) and *Heterobranchus 1ongifilis* (Clariidae). Aquatic Living Resources 8: 201 -206
Nwadiaro C. and P. Okorie, 1985: Feeding habits of the of the African Bagrid, *Chrysichthys filamentosus* in a Nigerian Lake, Japanese Journal of Ichthyology. 33: 376-383
Offem B. O., Y. Akegbejo-Samsons and I.T Omoniyi, 2009: Trophic ecology of commercially important fishes in the Cross River, Nigeria. The Journal of Animal and Plant Sciences 19: 37-44.
Offemin, B. O., Y. Akegbejo-Samsons, and I. T. Omoniyi. 2008. Diet, size and reproductive biology of the Silver catfish, *Chrysichthys nigrodigitatus* (Siluriformes: Bagridae) in the Cross River, Nigeria. International Journal of Tropical Biology. 56:1785-1799.
Ogwumba A. A. A., 1988. Food and Feeding habits of Juveniles of some culturable fish species in Nigeria. Technical Paper 31, Nigeria Institute for Oceanography and Marine Research. 24 pp

Osman, M. N. 1991. Interaction between dietary protein and carbohydrate levels in Tilapia Diets. Zagazig Veterinary Journal. 19: 627.

Oti, E. E. 2003. Studies on the ichtyofauna of Ehoma floodplain, Afikpo eastern Nigeria. ACP–EU Fisheries Research Report NO. 14. 9pp.

Padi J. N. 2006. Artificial reproduction of the freshwater fish, *Heterotis niloticus,* in experimental ponds in Southern Ghana. *Journal of the Ghana Science Association* 8: 45-53

Robinson, E. H., M. H. Li, and B. B. Manning. 2000. Evaluation of various concentrations of dietary protein and animal protein for pond-raised channel catfish, *Ictalurus punctatus*, fed to satiation or at a restricted rate. Journal of the World Aquaculture Society. 31:503-510.

Tacon, A. G. J. 1987. The Nutrition and Feeding of Farmed Fish and Shrimp. A Training Manual.1: The Essential Nutrients. A report prepared for the FAO trust fund GCP/RLA/075/ITA. Project support to the regional aquaculture activities for Latin America and the Caribbean.126pp.

United States Environmental Protection Agency (USEPA). 2002. Injurious Wildlife Species; Snakeheads (family Channidae). Federal Register: Volume 67, Number 193.

United States Geological Survey (USGS). 2010a. Literature review and background information. Accessed on May 20, 2010 at http://fl.biology.usgs.gov/Snakehead_circ_1251/html/literature_and_background.html.

Victor, R., and B. O. Akpocha. 1992. The biology of snakehead, *Channa obscura* (Gunther), in a Nigerian pond under monoculture. Aquaculture. 101(1-2):17-24.

Yang, S. D., C. H. Liou, and F. G. Liu. 2002. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). Aquaculture. 213:363–372.

Zhang, J., F. Zhou, L. Wang, Q. Shao, and Z. Xu. 2010. Dietary Protein Requirement of Juvenile Black Sea Bream, *Sparus macrocephalus.* Journal of the World Aquaculture Society. 41:151-164.

Prospects and Potential of the African Lungfish (*Protopterus* spp): An Alternative Source of Fishing and Fish Farming Livelihoods in Uganda and Kenya

Indigenous Species Development/Study/09IND07AU

John Walakira and Gertrude Atukunda
National Fisheries Resources Research Institute
Jinja, Uganda

Joseph J. Molnar
Auburn University
Auburn, Alabama, USA

ABSTRACT

Culture of species resilient to drought and stressed water quality conditions may be a significant part of the future of African aquaculture. Air breathing fishes potentially have a role in low-management culture systems for small farms because dissolved oxygen does not threaten the fish crop. The African lungfish (*Protopterus spp*) is advantageous because it is: an indigenous fish with good quality flesh, an air-breather, and a biocontrol agent against schistosome vector snails. African lungfish wild stocks in Uganda are falling, while no clear, sustainable strategies have been formulated to replenish the diminishing natural populations. Little is known about indigenous practices of culture, harvest, and marketing of *Protopterus spp* from farm ponds and water bodies.

Lungfish is highly valued as a food item in eastern of Uganda, and now is becoming more broadly accepted in the central region. Certain health or nutraceutical benefits are also attributed to the species. Most lungfish is consumed fresh but smoked products are also marketed. The fish is increasingly found alongside tilapia and Nile perch in some rural and urban markets. Nonetheless, there also seems to be some countervailing sociocultural beliefs that continue deter consumers from eating lungfish.
This study assesses the status and potential of lungfish aquaculture in Uganda in seven districts in Kampala, Wakiso, Kumi, Busia, Soroti, Pallisa and Jinja. Semi-structured interviews were conducted with key stakeholders; fish farmers, fisher folk communities, Fisheries officers, scientists, fish traders, and consumers. Socio-economic conditions (prices, demand, and public perceptions) that shape the culture of African Lungfish also were assessed.

INTRODUCTION

Globally, rising food prices have shifted 44 million people into extreme poverty while Uganda has nine million people facing an acute food shortage (World Bank, 2010). Aquaculture provides alternative source to food security and livelihood improvement, in the Sub-Saharan region population (Brummett and Williams, 2000). Lake fisheries continue to decline, and aquaculture production (subsistence and commercial oriented) is less than 1% of total national production (FAO, 2010; Isyagi *et al.*, 2009).
Fish is an important source of protein, contributing 42% of animal protein intake for 2.6 billion people, and, developing countries consider it as a human health asset (Brunner *et al.*, 2009). Increasing human demand for fish as natural wild fish populations are plummeting require urgent solutions (Brunner *et al.*, 2009). High prices of Nile perch and tilapia have reported shifted some consumers toward African lungfish, especially in densely populated areas of Kampala. In rural areas, the capita consumption of fish in general is reported to be as low as 1.9 kg (Nnyepi, 2006). Uganda has the some of the highest

population growth rates in central Africa but continues to have high incidences of chronic childhood malnutrition especially in rural areas (Owor *et al.*, 2000; Nnyepi, 2006; Nalwoga *et al.*, 2010). Aquaculture offers an alternative source to food security and livelihood improvement, in the Sub-Saharan region population (Brummet and Williams, 2000). Aquaculture in Uganda is mainly characterized by culture of Tilapia (*Oreochromis niloticus*), Catfish (*Clarias gariepinus*) and carp (*Cyprinus carpio*) at subsistence and semi –commercial levels; contributing less than 1% of total national production in each country (FAO, 2010; Isyagi *et al.*, 2009). Lungfish farming, therefore, will not only diversify farmed fish products in Uganda but will eventually reduce pressure on the declining stocks from the wild.

Shifting rainfall and temperature regimes are bringing new challenges to the management of water bodies and fish ponds in sub-Saharan Africa. The culture of species resilient to drought and stressed water quality conditions may be a significant part of the future of African aquaculture. Air breathing fishes such as the Marbled African Lungfish (*Protopterus aethiopicus*) are able to obtain and utilize oxygen from air to meet all or part of their metabolic demands. These fish are classified obligate air breathers, as adults of the species asphyxiate when denied access to air. They are carnivorous, eating crustaceans, aquatic insect larvae, and mollusks.

Air breathing fishes potentially have a role in low-management culture systems because dissolved oxygen is not a limiting factor. The African catfish (*Clarias gariepinus*) can tolerate low levels of dissolved oxygen but its flesh is often of less consumer interest. Concurrently, the *Pangasius* catfish's flesh is of high quality but is a species exotic to Uganda. Therefore, the African lungfish (*Protopterus aethiopicus* and *Protopterus amphibious*) is advantageous because it is an indigenous fish with good quality flesh, a biocontrol agent against schistosome vector snails (Daffalla *et al.*, 1985), and has some existing level of indigenous culture (Greenwood, 1966). The food value of the lungfish is enhanced by its high muscle to bone ratio, and its bones and cartilage pose less danger of choking consumers. Statistical estimates of per capita consumption of lungfish grew after 2000, but more recently declined to 6kg/year compared to global level of 12kg and the Sub-Saharan Africa of 7kg (DFR, 2011; FAO, 2011).

The African lungfish is native to the natural waters of Uganda (Birt et al., 2006; Greenwood, 1958, 1986) but rapidly declining, and, therefore endangered mainly due to its overexploitation, environmental degradation and the large-scale conversion of wetlands to agricultural land (Balirwa et al., 2003; Goudswaard et al., 2002). Catch trends of the African lungfish from Uganda waters suggest that quantities caught have stagnated for the past two decades with a peak during 1979 to 1985 period. Generally, there is a decline in the fishery from Uganda waters as quantities dropped from 411,800 metric tonnes in 2005 to 366,600 metric tonnes in 2010 (UB0S, 2010) according to statistics from the Uganda Bureau of Statistics (UBOS). And, yet the country has the highest population growth rates (3.2%) in world (UBOS, 2010; MFPED, 2010), that continues to exert pressure of its natural resources. Furthermore, insurgency in Northern Uganda caused migration to lungfish consuming regions of Uganda. A substantial number of affected people settled around lake regions of Kyoga, Bisina, Opeta and Victoria, most of whom who consume lungfish and regard it as a favorable food item. Thus, the lungfish seems to have broad consumer appeal in Uganda.

Physiology

Lungfish are members of the taxonomy class Sarcopterygii; they are lobe finned fishes (together with coelacanths).[1] The African lungfish is native to East African lakes, swamps, rivers and wetlands (Birt *et al.*, 2006; Greenwood, 1958, 1986). Lungfish are locally important food fishes captured from natural habitats in lakes and reservoirs using a variety of gear including gillnets, long lines, and other methods. It

[1] Order: Ceratodontiformes, Australian, S. American and African species Family: Protopteridae; Genus: Protopterus; there are at least four African lungfish species, Species: *Protopterus aethiopicus* (with three subspecies*), Protopterus amphibious, Protopterus annectens,* and *Protopterus dolloi* (Haeckel 1851).

is an endangered fish in Uganda as its natural stocks are rapidly declining mainly due to overexploitation, environmental degradation and the large-scale conversion of wetlands to agricultural land (Balirwa *et al.*, 2003; Goudswaard *et al.*, 2002). In Kenya's Lake Baringo, however, they dominate catches with annual landings of up to 90 metric tons after being introduced in 1970s and the fishery emerged in 1984 (Mlewa *et al.*, 2005; 2007; Mlewa & Green, 2004; Garner *et al*., 2006).

In nature, aestivating lungfish remain buried in mud cocoons relying solely on air to survive drought periods (lasting several months). Lungfish are periodically exposed to water with low oxygen content or situations into which their aquatic environment dries up. Their adaptation for dealing with these conditions is an out pocketing of the gut, related to the swim bladder of other fishes, which serves as a lung. The African lungfishes are obligate air breathers, with reduced gills in the adults. 2

African lungfish breed at the beginning of the rainy season. They construct nests or burrows in the mud to hold their eggs, which they then guard against predators. When hatched, the young resemble tadpoles, with external gills, and only later develop lungs and begin to breathe air (Goudswaard *et al.*, 2002).

Basic work on pond culture by Mlewa *et al.* (2009) finds indications of early breeding behavior, as the trial lungfish attained sexual maturity slightly earlier than those in wild populations since the lungfish that made burrows and were not accessible for harvest. Culture trial results showed that lungfish realized growth increments of 2.7 and 14.5 cm over time periods ranging from 70 to 238 days. The mean absolute growth rate was 0.049 (±0.008 SE) cm/ day, whereas specific growth rates ranged from 0.048 to 0.140% per day. This study demonstrated that marbled lungfish can be raised in earthen ponds and suggested that further research determine its potential in the aquaculture industry. Furthermore, efforts to develop culture techniques must also address handling and harvesting issues associated with a fish that has a "beak" like a snapping turtle and a tendency to burrow in the soil when a pond is drained (Mlewa *et al.* 2009).

Traditional Practice

Local practice is to excavate lungfish, burrow and all, and store it for later use when they want fresh fish to eat. As use of long lines and gillnets are increasing, Uganda lake and river lungfish populations are decreasing. In Uganda, some women do not eat lungfish under the belief that it is a "sister fish," associated with men and manhood (Bruton, 1998).

Little is known about indigenous practices of culture, harvest, and marketing of *Protopterus spp* from farm ponds and water bodies. Anecdotal evidence suggests farmers gather wild nestlings of lungfish and stock small water bodies but with no documentation of management practices or yields (Mwatete *et al.*, 2005). Preliminary attempts in Kenya to grow wild Marble lungfish (*Protopterus aethiopicus*) 'fry' in earthen pond encountered difficulties because most fish went into burrows and disappeared.

Culture trial results undertaken by Mlewa *et al.* (2009) with African lungfish showed growth increments of 2.7 and 14.5 cm were realized over time periods ranging from 70 to 238 days. The mean absolute growth rate was 0.049 (±0.008 SE) cm/ day, whereas specific growth rates ranged from 0.048 to 0.140% per day (Mlewa *et al.* 2009). This study demonstrated that marbled lungfish can be raised in earthen ponds and suggested that further research determine its potential in the aquaculture industry. Baer *et al.* (1992) succeeded in culturing wild-caught *Protopterus amphibius* juveniles grown in concrete tanks at a

[2] They have two anterior gill arches that retain gills, though they are too small to function as the sole respiratory apparatus. The lungfish heart has adaptations that partially separate the flow of blood into its pulmonary and systemic circuits. The atrium is partially divided, to that the left side receives oxygenated blood and the right side receives deoxygenated blood from the other tissues. These two blood streams remain mostly separate as they flow through the ventricle leading to the gill arches. As a result, oxygenated blood flows mainly to the anterior gill arches and the deoxygenated blood flows to the posterior arches (Goudswaard et al., 2002).

density of two fish per m^2. They obtained good results with fish fed with soft balls containing raw minced beef heart and cooked tilapia.

Efforts to develop culture techniques must also address handling and harvesting procedures associated with a fish that has a "beak" like a snapping turtle and a tendency to burrow in the soil when a pond is drained. Lungfish have sharp plate-like that are not well developed like other types of fish and uses them to feed generally on live fish and mollusks. Its teeth are sharp making dangerous to handle that incidence of some fishermen have lost a finger to lungfish attack. Fishermen use baits (mainly *Clarias* spp) and papyrus made baskets to trap it but use hoes or spears to hit it its head to avoid being hurt or bitten. Baer *et al.* (1992) explains how frequent handling of lungfish stresses the fish leading to aggressive actions, and gentle handling reduced the application tranquilizers. Interestingly, fish farmers have succeeded in seining out lungfish from their ponds but still have to use spears to kill or hoe to kill. Implying that lungfish can be harvested using available gears, nevertheless appropriate technologies will have to be developed to address aquaculture perspectives.

Lungfish ferociously protect their eggs and nestlings which makes it difficult to collect fry or nestling for fish farming. Furthermore, lungfish fingerling rarely swims in schools as observed by many fishermen. Fingerlings are normally seen swimming under mats of water hyacinth (*Eichhornia crassipes*) and around papyrus vegetation which makes it difficult to collect large numbers, for example in thousands. However, Mlewa *et al.* (2009) revealed indications of early breeding behavior, as the trial lungfish attained sexual maturity slightly earlier than those in wild populations since the lungfish that made burrows and were not accessible for harvest. Therefore, it may be possible to develop breeding techniques for lungfish in captivity.

Surveys undertaken by the National Fisheries Resources Research Institute (NaFIRRI) indicate the majority of caught using gill nets and hooks are in stage IV-V which is a mature fish (NaFIRRI, 2005, 2006, 2007) which may indicate a fishery under pressure as the recruitment process is interfered with. The sharp decline is attributed to the increase in population and number of fishing boats. Immature fish is also caught and sold in the markets regardless of stringent regulatory policy in place. Interestingly, the existence of 'immature' females from the wild that have mature eggs is a major challenge to current fisheries regulations to protect immature lungfish.

The literature on African lungfish mainly examines lungfish ecology, fishery, biology, and physiology, but few studies treat its use as a food fish in aquaculture (Baer et al., 1992). Therefore, this study applies what is known about lungfish to explore its aquaculture potentials in improving food security and livelihoods in sub-Saharan Africa. The study assesses indigenous practice and understandings about production parameters and approaches. The field work assesses potential paths for producer adoption and training to use lungfish as a culture species and a managed water body resource.

METHODS AND MATERIALS

The first part of this study involved collecting and collating existing information from government fisheries departments; the Department of Fisheries Resources under Ministry of Agriculture, Animal Industry and Fisheries, District Fisheries headquarters and the National Fisheries Resources Research Institute (Jinja and Kajjansi). Discussions were held with government officials on policy towards lungfish in the wild and in aquaculture, as well as market trends. This information was further supplemented with publications or reports from government agencies.

Field Visits

The second part involved gathering primary information obtained through conversations with fishers, marketers, and consumers about the potential for lungfish culture in Uganda. Informal discussions were

held in eight districts (Kampala, Wakiso, Mukono, Kumi, Busia, Soroti, Pallisa and Jinja). These were districts known to have some level of indigenous practice with lungfish. Local field extension workers provided translation where local languages were spoken (Figure 1). We spent more time with those capturing and marketing lungfish and less time with consumers. We met people at fish farms (34), fish landing sites (10), fish markets (10), restaurants (17) and visited government fisheries offices (11) (Table 1).

Fish farmers, residents of fisher communities, district extension officers, fish traders and scientists were contacted to assess indigenous knowledge and practices associated with the culture and use of lungfish on farms in ponds, in natural water bodies and reservoirs. Guiding questions for the discussions centered on reasons for involvement with lungfish, harvest and handling practices, and fish farming and when it started, problems encountered, and overall views on lungfish.

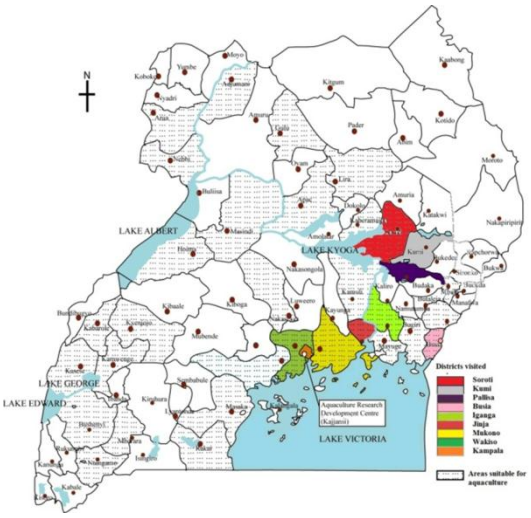


Figure 1. Map of Uganda showing study locales.

We attended fish markets to assess lungfish prices, demand, and public perceptions. We visited fish markets reputed to trade in African lungfish, located within city areas or in proximity to fish landing sites. Lungfish growers, fishers, restaurant owners, consumers, fish traders, wholesalers and retailers were key informants. We asked questions about types of fish supplied or sold, quantity, prices, seasonal variations, and gender participation.

Study districts

Eight districts were purposively selected for field work. We relied on information provided by fisheries officers and administrative records to select study areas. Each is located in areas or zones suitable for aquaculture production and each had reported markets for lungfish.

Table 1. Number of contacts by type and district, Uganda lungfish study, 2011

District	Fish Farmers	Landing sites	Fish Traders	Consumers	Restaurants	Government Institutes*
Soroti	5	1	7	16	2	1
Kumi	6	2	10	11	1	1
Pallisa	2	1	2	5	0	1
Busia	5	1	5	9	1	1
Iganga	0	0	2	0	1	0
Jinja	5	2	6	16	3	2
Mukono	2	0	1	2	2	0
Wakiso	8	2	9	11	3	3
Kampala	1	1	23	24	4	2
Total (n)	**34**	**10**	**65**	**94**	**17**	**11**

*Institutes include District Fisheries Office, National Fisheries Resources Research Institute and Department of Fisheries Resources Headquarters

RESULTS

Lungfish in East Africa

Most lungfish is captured in Uganda's natural waters; contributing over 90% of lungfish caught in the three Lake Victoria countries (Kenya, Uganda and Tanzania). In the period 1975 to 2009, a total of 404,008 tons of lungfish have been caught of which 371,811 tones were caught in Uganda and 32,197 tones captured in Kenya (Figure 2). No records show lungfish caught in Tanzania.
Uganda recorded the highest quantity caught (15,000-22,000 tons) during 1976 to 1985, then decreased during 1985 to 1989, with steady production thereafter. The amount of Lungfish caught in Kenya has stagnated around 1000-3000 tons in the four decades. However, from 2005 onwards statistics generally show a decline in catches.

Figure 2. Lungfish in the market.

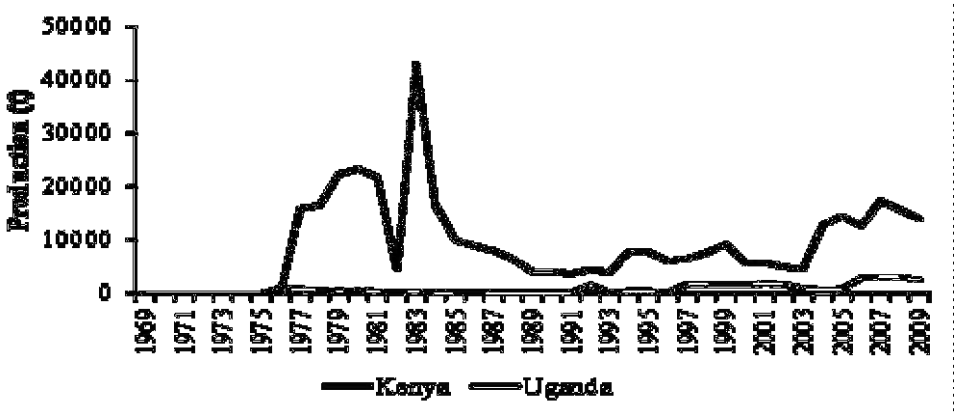


Figure 3. Capture trends of Lungfish caught in Kenyanya, Tanzania and Uganda. Sourced from FAO.

Fisheries of Uganda

Overall, lungfish contributes about 4% of the total fish caught from natural water bodies in Uganda. Tilapia (37%) and Nile perch (42%) are the largest quantity harvested (Figure 3). Lungfish are mainly caught using gill nets, hooks, basket traps, or long-lines.

Nile perch and tilapia catches steadily increased in the period 1969 to 2006, but started declining afterwards. Peak catches of lungfish were seen in the period 1977 to 1983. Thereafter, lungfish catches declined for about six years until 1989 when the quantity stagnated or leveled in thousand tones.

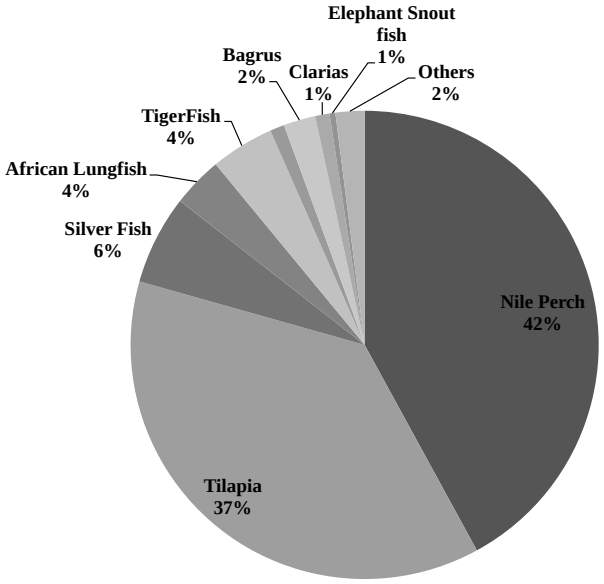


Figure 4. Main fish species caught in Uganda waters (1999).

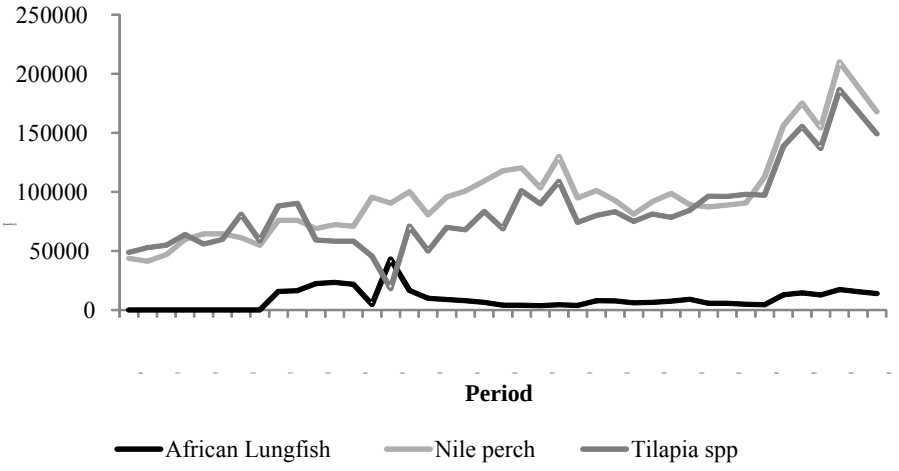


Figure 5. Trends of Fish (Nile perch, Tilapia and African Lungfish) caught in Ugandan Lakes. FAO.

Market Sources

African lungfish is constantly being hunted even during the dry season when it is aestivating. Most lungfish is caught from the wild environments (lakes, swamps or seasonal rivers) and either sold in local markets or consumed directly at home. Breeding sites for African lungfish are disappearing as wetlands are converted to farm land to produce food. Variations in catches occur across districts visited as Kumi and Soroti have the largest quantity caught from Lakes Opeta, Bisina, Nyaguo and Kyoga. Other districts obtain lungfish from Lake Victoria.

Figure 6. A) Fishers at Akidel landing site in Kumi district; B) Size variation of lungfish caught from Lake Opeta.

Over 50% of lungfish (locally known as *Ebileng*) in Kumi district is mainly from Lakes Opeta and Bisina at Ometanga and Akidea landing sites, respectively. Records from Lakes Opeta, Bisina and Nyaguo, taken since 2005, show that lungfish contributes 59% of the total fish harvest, while tilapia (18%), *Clarias spp* (18%), and other types of fish (5%) are also caught (figure 4). High catches of lungfish occur during low tides and the onset of rainy seasons. Nonetheless, most fish are 'immature' or small sized.

Fishermen report that some young female lungfish of sixe 300 grams taken from the wild have mature eggs. Also, according to district officials and residents of fisher communities, the size and numbers of lungfish caught is falling. In the early 1990s, catches were over 3,000 tons per year but recent government records show an annual total of 154 tons. Additionally, over 400 fishing boats are used on the lakes and utilized in turns (day and night), to increase fishing efforts.

Two forms of lungfish are harvested, *eigolo*, the giant lungfish and *ebilongotuba*, a tailless form that is harvested while aestivating in the dry season. Records at landing sites in Busia, Jinja, Wakiso and Kampala districts show that lungfish landings are third after Nile perch and tilapia

There are no clear guidelines to restrain the taking of small lungfish. Most captured lungfish reportedly weight less than a kilogram. In Soroti, lungfish is sourced from Lakes Kyoga and Opeta. However, the average size of lungfish harvested from Lake Kyoga is larger than those caught from Opeta and other small lakes in eastern Uganda.

Evaluating lungfish as a culture species

A total of 34 fish farmers were contacted in eight districts; Soroti, Kumi, Pallisa, Busia, Jinja, Mukono, Wakiso and Kampala. All culture systems visited were earthen ponds averaging 300 m^2. Main fish

stocked include Nile tilapia (Oreochromis niloticus) and the African catfish (*Clarias gariepinus*), cultured in either polyculture or monoculture systems.

Only one farmer in Nangabo (Wakiso district) had stocked ponds with lungfish since 2003. He stocked 1,000 juveniles (length, 15-20 cm) together with Mirror Carp and tilapia in a 400 m^2 pond. He fed the fish with maize bran mixed with mukene (fish meal), once every day for eight months. No proper records were kept to determine the amount fed daily. Fish were kept in ponds for 1.5 years, but yielded 361 adult lungfish that ranged one to three kilograms. He sold the lungfish in Kampala fish markets for about US $ 2.5 – 4.0 per kg. Unfortunately, he abandoned fish farming in 2006 because he was too old to run the farm.

No other fish farmers stocked African lungfish. However, some reported harvesting lungfish from their ponds in areas that are frequently prone floods, especially in Kumi and Soroti. These fish farmers attribute the loss of stocked fish in their ponds to the predation of lungfish from the wild, and, their burrowing habits that cause leaks. One Soroti district farmer claimed to have lost over 70% of the stocked catfish after discovering adult lungfish in his ponds. Furthermore, some farmers reported the presence of lungfish created turbid waters in their ponds. Nevertheless, lungfish found in fish ponds are either consumed at home or sold in nearby markets.

Overall, about 56% of the fish farmers (N=34) were willing to grow lungfish, while 41% where not willing for various reasons. Three percent said they had abandoned fish farming altogether. Those who were willing; Kumi district (28%) had the highest interest then, Soroti (22%), Busia (22%), Wakiso (17%) and Jinja (11%).

The main reasons farmers gave for engaging in lungfish farming were: the availability of markets and good prices (71%); the fish product quality—it does not smell and has a substantial fillet size (23%); and others mentioned the large size—lungfish may exceed one kilogram (6%). Surprisingly, one farmer in Busia had his ponds ready prepared for stocking lungfish fingerlings that he will was going to obtain from shores of Lake Victoria. He felt he could use his catfish-rearing experience to succeed in growing lungfish.

Fish farmers who were not willing to undertake lungfish farming where mainly from the central districts; Jinja (28%) and Wakiso (27%). Others were from Pallisa (18%), Busia (9%), Soroti (9%) and Kampala (9%). The main reasons given were religious and tribal beliefs (36%), centrally concerning attributions of negative impacts on female consumers. Other reasons for not growing the fish related to predation (22%), lack of technical guidance on how to culture lungfish (14%), concerns about its burrowing habits (14%), doubts about its market value (7%), while others had no knowledge of the fish (7%).

Several previous attempts to culture lungfish were reported. In Kumi, some fishermen living around Lake Bisina and Opeta stocked 15-30cm juveniles in excavated holes (40cm diameter; 1m deep). They fed these small batches daily with fish fry (mainly tilapia and catfish), grasshoppers, snails and food trash. No attempts to feed plant materials were reported. After a year, the lungfish reached about 70 cm in length, they were harvested using hooks. According to these fishermen, some lungfish were lost to cannibalism.

Others escaped harvested because they burrowed into pond soils.
In 2009, a fish trader in Bwaise (Kampala) attempted to raise a juvenile (>20cm) in a concrete tank (1x 1 x 0.5 m^3). He fed it with food trash or leftovers from home, harvesting after seven month when the water turned green and smelly. He was disappointed with the 60 cm. average size of the harvested lungfish.

Feeding African lungfish with fry of tilapia and Clarias spp shows that the fish can be polycultured with prolific Tilapia spp. This may be possible if the lungfish is the targeted as the primary cultured fish

because as a carnivore it decimates most of the tilapia population. Raising it in cylindrical dug-holes and concrete tanks provides us with an insight on which culture systems can be used to raise. Lungfish can burrow through pond dykes leading to leaks, and at times of loss of fish. Some advantage could accrue to raising the species in concrete tanks or ponds. One Bwaise trader reported trying to raise lungfish in a concrete tank, but had water quality problems that apparently stunted the fish. Therefore, in culturing lungfish water quality may need to be studied.

Consumption of fish

Per capita fish consumption in Uganda increased from 2000 to 2007, but gradually declined to 6 kg afterwards (DFR, 2011). Most respondents consider fish to be an expensive protein commodity; as result people tended to reduce the amount regularly consumed. Many attribute high fish prices to scarcity from the wild. Most lungfish is consumed fresh, but cured products (smoked and sun dried) also are available in markets.

Diverse views of lungfish as a food item were obtained across the eight districts visited. Those who regularly consume lungfish came from eastern districts (55%)--Kumi, Soroti and Busia. Among the central districts, Kampala (21%) had the highest numbers of consumers of lungfish, in contrast to Wakiso (12%) and Jinja (12%). The main reasons consumers gave for preferring lungfish were: price was cheaper compared to other fish species (Nile perch and tilapia); the taste and fillet size is good and adequate to feed an average family; and for attributed medicinal (treatment and prevention) properties.

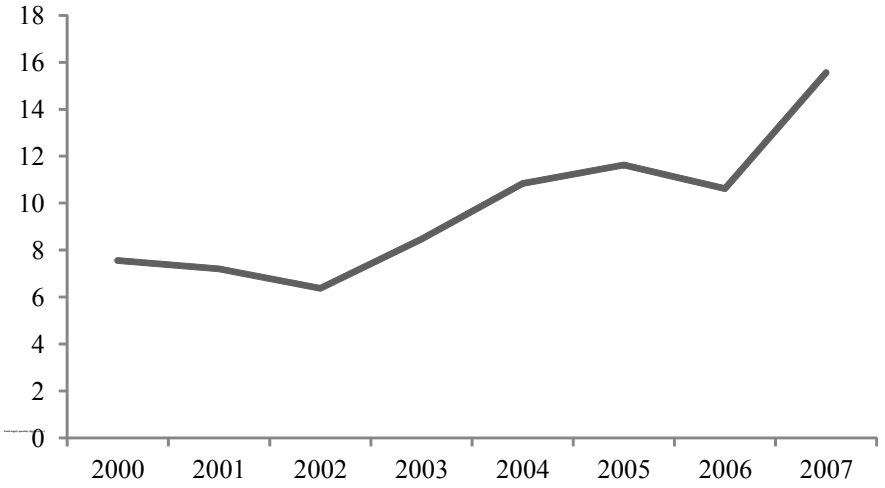


Figure 2. Per capita fish consumption in Uganda from 2000 to 2007. Sourced from FAOSTAT

Lungfish was most highly valued in Kumi and Soroti districts. In the villages, lungfish is often considered a delicacy or special dish that is normally prepared for in-laws. Lungfish seem acceptable to both women and men. Most seem to prefer fresh lungfish, but smoked and salted forms also are found in markets (figure 6). In Busia district, demand for lungfish (locally known as *Emonye*) is high but supply was reported to be low.

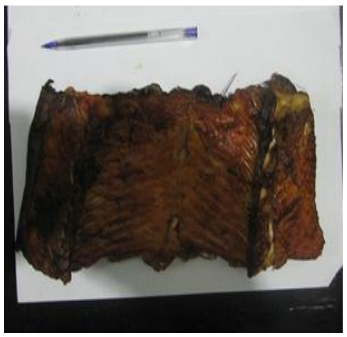
In Pallisa, lungfish is known as *Nakibalo or Mamba* mainly caught from Lake Lamwa. It is not popular amongst the Bagwere communities. It is popular at the landing sites were the Iteso form the majority residents. Central region had the highest number of respondents (82%) who had never eaten lungfish, but far fewer in Wakiso (35%) and Kampala (23%). A few respondents in the eastern region Pallisa (9%) and Busia (9%) had not eaten lungfish; but none in Kumi and Soroti. Main reasons given for not eating

lungfish were: tribal or traditional beliefs that restricts them from eating lungfish (61%); no prior knowledge about the fish (25%); religious beliefs about 'scale-less' fish (11%); and others could not eat it because of its external appearance (3%).

A few respondents came from Mbale district where lungfish is called *Nambere*. In that region lungfish are mainly obtained from Namatala and Mpologoma streams around Lake Nakuwa. In Mbale, lungfish is not popular among the Gisu community to the extent that cooking utensils were separated if used to prepare lungfish.

In this study over 70% of the respondents were female. About 68% report having eaten lungfish. Again, the majority come from the eastern region districts of Kumi and Soroti. One striking difference observed is in Kampala district where over 60% of female respondents eat lungfish. Lungfish also is sought for its ascribed benefits for human health. It is used to treat women's breasts for problems of lactation; the fish's pancreas is reportedly used to treat alcoholism. The tail is used to enhance male's sexual performance. Eating lungfish is believed by some to boost the immune system.

Smoked piece of lungfish



Fresh lungfish gutted

Figure 8 (left). Lungfish products observed in local markets

Figure 9 (right). Lungfish market in Bwaise, Kampala.

Restaurants

Only a limited number of restaurants in the study locales have lungfish on their menu. Only six (35%) restaurants visited prepared lungfish for their customers. Smoked and fried products where the main dish sold in restaurants. However, restaurants in Jinja and Busia reported that lungfish is normally prepared on prior request by customers. Some restaurants or Bars in Kabusu and Owino in Kampala have specialized in selling fried African Lungfish to its customers. Most customers like African lungfish meat because it is not fatty, does not smell like Nile perch and is satisfying.

Lungfish markets

Fish traders play an important role in mediating lungfish fishers, farmers, and consumers. Wholesale prices for fresh lungfish range between US $ 0.9 to 1.80 per kg while retail prices can go beyond US $ 2.5 per kg depending on the location. Price for cured products (smoked) range from US $ 3 – 4 per piece. Prices of lungfish are relatively lower in rural areas than in populated towns or cities. Also, juveniles of about 15cm total length are treated as by-catch and marketed in clusters. Others are prepared and eaten fresh after being gutted.

In some Kampala suburbs, kiosks that used to sell Nile perch products commonly known as “fille” are now substituting it with African lungfish. Low income residents, particularly youth are regular customers. Majority kiosks selling fried lungfish are owned by women who procure the fish from major fish markets in Kawempe-Bwaise (Kampala) and Busega (Wakiso). Some women have taken loans to initiate their lungfish businesses. Fish traders play an important role in mediating lungfish fishers, farmers, and consumers.

Information derived from this study on consumers’ perspective may not be conclusive but we see a change in life style in the consumption towards lungfish. Bruton (1998) reports that some women do not eat the lungfish because they consider it a "sister fish", with some undesirable consequences for the female consumer. In this study we observed not only active participation of women in lungfish trading but also consuming it. Some districts had low rates of lungfish eater among women and men especially in the central region where it is not customary consumed by some clans. Nevertheless, the field observations suggest increasing interest in lungfish consumption, locally and regionally.

Harvesting and handling

Farmers normally harvest the lungfish after completely draining the pond. Others have ever harvested lungfish using pond seine nets. To identify the presence of lungfish in the pond, farmers trace clear waters along the bank and extract it from the holes using hoes and spears. In Soroti and Kumi, farmers detect the presence of lungfish in burrows by tying a tuft of grass around their legs or stick, which the fish seizes with its plate like teeth. As it holds on to bait its extracted from the hole, slowly, hit on the head using a hoe or spear. Other farmers also use baits on hooks usually *Clarias* spp and earthworms caught from the wild. The *Clarias* spp bait is the most effective bait. It is easy and safer to harvest them during the dry season because they are relatively inactive. Lungfish harvested is handled around the neck avoiding the mouth parts.

Figure 10 (left). Lungfish nest with fry along shore of Lake Opeta, Uganda.

Figure 11 (right). Eggs extracted from a female lungfish.

Hooks and basket traps (locally called *Ekolo*) are mainly used to catch the lungfish in seasonal wetlands. In the dry season (December, January and February) lungfish is dig-up from holes using hoes when the local communities cultivate or hunt around the wetlands. Women play a major role in lungfish fisheries; hunting, post harvesting processes and the marketing. In Pallisa, lungfish is locally known as *Nakibalo or Mamba*. The fish is mainly caught from Lake Lamwa using hooks number 5, gill nets of 4 to 4.5 inches and baits used include pieces of meat, rats, and frogs. In Busia lungfish is sourced from the shorelines of Lake Victoria and swampy areas using hooks (number 12), basket traps and spears.

Identifying simple fingerling production techniques

No established procedures have been yet been developed to produce lungfish fingerlings. All fingerlings were acquired from the wild, mainly from mats of water hyacinth (*Eichornia crasipes*) and in the nests. Furthermore, obtaining a substantial number (more than 1000s) may be difficult as many lungfish fingerlings are reported to swim individually rather than in schools. However, fishermen can time breeding seasons of lungfish when the fry or fingerlings are available; during the onset of rains.

It is difficult to distinguish males from males lungfish because they appearance is similar but some speculate orientation of genital opening beneath the pelvic fin may correlate with the sex of the fish. The female genital opens on the right while the male opens to the left. Some fishermen did not agree to this revelation because at times fish that is thought to be a male normally has eggs when gutted.

The female produces very many eggs and lays them in stagnant water in a hole or a nest away from sunlight and guards them. The eggs are deposited on the base of the nest and the male fertilizes them later. It is not known how long it takes for the eggs to hatch in such conditions. It is very difficult to get fertilized eggs or fry from the nest because the lungfish ferociously guards the nest. However, one can extract eggs from a wild caught female.

CONCLUSION

The study assessed indigenous practice and understandings about lungfish as a potential culture species in Uganda and more broadly in Sub-Saharan Africa. Fish farmers already have inadvertently farmed lungfish that entered their ponds during flood periods. An experiment program in needed to establish production parameters as little is known about the growth cycle and nutritional needs of farm-reared lungfish. For example, optimal water temperatures, salinity tolerance, and other basic parameters of the species are not known. It is understood that they survive and grow alongside tilapia, for example, but optimal feed composition and lungfish grow-out strategies remain to be articulated.

At present, growers are reliant on wild-caught lungfish fry for what limited culture is currently taking place. Research must clarify the reproductive cycle of the lungfish to enable farm-based spawning and seedstock production of uniform batches of genetically advantaged fish. A clear foundation for establishing an industry, the biology and manipulation of lungfish reproduction processes is not well-understood.

Farmers have developed indigenous means for handling and managing lungfish in natural water bodies and farms ponds. These are a beginning to be discovered and codified. Promoting wider levels of production of lungfish will require articulation of model production strategies and management systems that account for the burrowing and mobility of lungfish. Clearly, cage culture would overcome some of the known difficulties, but this work has not yet been accomplished.

Lungfish is a delicacy among groups in the Northern, Eastern and some parts of western Uganda. Thus the present and potential consumer demand for the species is fairly well-established. The field work assessed potential paths for producer adoption and training to use lungfish as a culture species and a managed water body resource.

Lungfish may be raised on artificial diets as all fish farms that had the fish in their ponds applied commercial pellets to catfish or tilapia stocked. Efforts to domesticate African lungfish are foundational to the advance of a commercial industry providing a valuable food item to people in need of affordable protein.

This study shows how initiatives to culture the fish build on indigenous knowledge and practice to formulate a broader strategy of widespread production. Future studies will explore the relative advantages of different culture systems (tanks, ponds and cages), while addressing specialized procedures for grow-out and harvest.

The socioeconomic viability of African lungfish as a new culture species is beginning to be established. This report identifies the central issues of reproduction, feeding, and management that must be addressed in order to build an industry with a value chain that delivers quality products to consumers and a sustainable return to small- and medium-scale producers in Uganda and across Sub-Saharan Africa.

ACKNOWLEDGMENTS

This work is being supported AquaCRSP under the USAID. We thank Karen Veverika (Auburn University) who provided technical guidance. Special thanks to government fisheries technocrats and Maureen Kaitesi (Research assistant) for guiding the investigating team.

LITERATURE CITED

Baer, S., Haller R. D., Freyvogel T. A. (1992). Growth of the African lungfish, Protopterus amphibious Peter, in aquaculture. Aquaculture Research 23, 2, 265-267.

Balirwa, J.S, Chapman C. A, Chapman L.J, Cowx I.G, Geheb K, Kaufman L, Lowe-McConnell R. H, Seehausen O, Wanink J.H, and Welocomme R.L and Witte F. (2003). Biodiversity and fishery sustainability in the Lake Victoria Basin: an unexpected marriage? BioScience, 53(8):703-716. 2003.

Birt, T.P, Mlewa. C. M, Green M, Seifert. A. and Friesen V. L. (2006). Genetic variation in the marbled lungfish *Protopterus aethiopicus* in Lake Victoria and introduction to Lake Baringo, Kenya. J of Fish Biology 69, 189-199.

Boyd, C. E., S. Soongsawang, E. W. Shell, and S. Fowler. (2010). Small impoundment complexes as a possible method to increase water supply in Alabama. Proceedings of the 2009 Georgia Water Resources Conference, University of Georgia, Athens, Georgia, USA.

Brummett R.E and Williams M. J, (2000). The evolution of aquaculture in African rural and economic development. Ecological Economics 33 (2) 193-203.

Brunner E. J, Jones P. J.S. Friel. S and Bartley M. (2009). Fish, human health and marine ecosystem health: policies in collision. International Journal of Epidemiology 38 (1):93–100.

Bruton, Michael N. (1998). In Paxton, J.R. & Eschmeyer, W.N.. ed. Encyclopedia of Fishes. San Diego: Academic Press. pp. 70–72. ISBN 0-12-547665-5.

Daffalla, A.A., Elias E.E., Amin M.A. (1985). The lungfish Protopterus annectans (Owen) as a biocontrol agent against schistosome vector snails. J Trop Med Hyg. Apr 88(2):131-4.

Dunbrack, Green & Mlewa. (2006). Lungfish growth - Journal of Fish Biology 68, 443-447.

FAO. (2010). The State of World Fisheries and Aquaculture. 2010. Rome Italy.

Garner, S., T. P. Birt, C. M. Mlewa, J. M. Green, A. Seifert, V. L. Friesen. (2006). Genetic variation in the marbled lungfish Protopterus aethiopicus in Lake Victoria and introduction to Lake Baringo, Kenya. Journal of Fish Biology 69(b), 189-199. Retrieved September 22, 2011 http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.2006.01224.x/full

Goudswaard, K.P.C., F. Witte, and L. J. Chapman. (2002). Decline of the African lungfish (*Protopterus aethiopicus*)in Lake Victoria (East Africa). Afr. J. Ecol., 40, 42-52.

Goudswaard, Kees, Frans Witte, Lauren J. Chapman. (2002). Decline of the African lungfish (*Protopterus aethiopicus*) in Lake Victoria (East Africa) East African Wild Life Society, African Journal of Ecology, 40, 42-52.

Greenwood, P. H. (1966). The Fishes of Uganda. The Uganda Society, Kampala.

Greenwood, P.H. (1958). Reproduction in the East African lung-fish *Protopterus aethiopicus* Heckel. Proc. Zool. Soc. London 130, 547-567.

Greenwood, P.H. (1986). The natural history of African lungfishes. J. Morph. Suppl.1,16 3-179. http://www.ubos.org/onlinefiles/uploads/ubos/pdf%20documents/2010StatAbstract.pdf. Retrieved 1st September 2011.

Isyagi N, Atukunda G, Aliguma L, Ssebisubi M, Walakira J, Kubiriza G, Mbulameri E (2009). Assessment of national aquaculture policies and programmes in Uganda. Aquaculture Compendium. 87 pp.

Mlewa and J. Green (2004). Biology of the Kamongo African Journal of Ecology 42, 338-345.

Mlewa, C.M. & J. Green (2006). Introduction and Fishery - African Journal of Aquatic Science 31, 131-136.

Mlewa, C.M. C.M., Green & Dunbrack (2007). Lungfish respiration in the wild. African Zoology 42, 131-134

Mlewa, C.M., Green and Simms (2005). Movement and space Use. Hydrobiologia 537, 229-238.

Mlewa, C.M., J. Green & Dunbrack (in Press) Natural history (Book Chapter: Jorgensen & Joss (Eds).

Mlewa, M. C., Ogola, D. W. and Ngugi, C.C. (2009). Aspects of the biology of marbled lungfish (Protopterus ethiopicus) raised in earthen ponds at the Chepkoilel fish farm, Kenya. Journal of East African Resources Management 16: 231-34.

Moehl, J., M. Halwart, and R. Brummett. (2005). Report of the FAO-World Fish Center Workshop on Small-scale Aquaculture in Sub-Saharan Africa: Revisiting the Aquaculture Target Group Paradigm. Limbé, Cameroon, 23-26 March 2004. CIFA Occasional Paper. No. 25. Rome, FAO. 2005. 54p. Retrieved 14 January, 2009: http://www.fao.org/docrep/008/a0038e/a0038e04.htm#TopOfPage.

Mwatete, M. C., Ogola, D. W., and Ngugi, C.C. (2005). Towards the recruitment of marbled lungfish (Protopterus ethiopicus) in aquaculture in the lake Victoria basin: some preliminary results of culture trials at the Chepkoilel fish farm, Kenya. 2nd National Scientific Conference, Lake Victoria Environmental Management Project. 17th to 19th October 2005. Tom Mboya College, Kisumu, Kenya.

UBOS. (2001). The 2000/01 Ugandan Demographic and Health Survey. Kampala: UBOS

UBOS. (2002). Uganda DHS EDU Data Survey 2001. Kampala: UBOS.

Wikipedia. (2011). Protopterus. Retrieved May 14, 2011, http://en.wikipedia.org/wiki/Protopterus

World Bank. (2010). Global Economic Prospects 2010: Crisis, Finance, and Growth. Washington, DC (www-wds.worldbank.org).

Effects of Environmental Conditions on Gills and Gas Bladder Development in Bimodal-Breathers, Gar (*Lepisosteus* sp.), Pirarucu *(Arapaima gigas*) and Bowfin (*Amia calva*)

Indigenous Species Development/Activity/09IND08UH

Konrad Dabrowski
Ohio State University
Columbus, Ohio, USA

Maria Celia Portella
Sao Paulo State University
Jaboticabal, Brazil

Leszek Satora
Jagiellonian University
Krakow, Poland

ABSTRACT

The long-term goal of this research was to increase our understanding of phenotypic adaptations to changes in oxygen concentration, beyond sustaining growth. The growth response is implicitly related to the controlled aquaculture conditions, however the predictive value of oxygen saturation on growth can be extended to the interpretation of the results in the ponds and estuaries where variation in oxygen saturation are diurnal and seasonal. The design of the experimental matrix allowed us to distinguish between several components of the response such as basic morphological changes, behavioral adjustments, and in some cases, growth.

We have performed one experiment with bowfin (*Amia calva*) and one with prirarucu (*Arapaima gigas*). We further analyzed gill structure of spotted garfish (*Lepisosteus oculatus*) that was studied earlier. The first artificial propagation of bowfin performed after hormonal induction confirmed timing of embryonic development, hatching, yolk absorption and first exogenous feeding as has been described in the literature. Fertilization was carried out and mass hatching occurred after 8 days at 16^{o}C. The larvae were fed live *Artemia* nauplii and then weaned to a formulated starter feed at a size of 25 mm. The fish were grown in glass aquaria at 18-22^{o}C. They were divided into groups of 40 individuals and conditions were set up at normoxic, 90% oxygen saturation, hypoxia (35%) and hyperoxia (170%). There were 4 replicates for each treatment. The growth rate was monitored during 30 days and no significant differences were found. There were significant differences in frequency of air gulping between treatments, 25 $\pm$ 2 per hour per fish in hypoxia, 58 $\pm$ 8 in normoxia and 84 $\pm$ 4 in hyperoxia. Fish were sampled for histological analysis of gills and gas bladder structure at the termination of the exposure studies.

In the case of pirarucu, the experiment included four replicate treatments of juvenile pirarucu at acute hypoxic and hyperoxic conditions for 8 hours, followed by the return to normoxia for 10 h. During the exposure frequency or air gulping was observed at 1 h intervals. When the stress effect during the first hour after transfer to experimental conditions was not included, there was a significant difference in air gulping frequency between hypoxia (3 $\pm$ 1 per 3 min) and hyperoxia (1 $\pm$ 0.5). No mortality or other stress signs were observed. Fish were sacrificed after hypoxia/hyperoxia exposure and 10 h after recovery in normoxia for histological examination of gills and gas bladder.

OBJECTIVES

Our primary experiments were designed to investigate the following:

1) Determine morphological structure of gills and respiratory bladder in young-of-year garfish and pirarucu.
2) Determine ontogenetic changes in the development of gills and respiratory bladder in bowfin larvae and juveniles in order to establish a model species for air-breathing fishes.

INTRODUCTION

Garfish

Dissolved oxygen level affects fish performance and species able to use or are dependent on atmospheric air for respiration hold great advantage in frequently occurring conditions of hypoxia in aquatic environment over non-air breathing teleost fish (Diaz, 2001). If we accept the premise that hypoxia is more frequent in the tropics and that global climate warming will lead to aggravation of hypoxia in temperate climatic zone, air-breathing species of fish are particularly amenable to farming for human consumption.

Fishes throughout their evolutionary history acquired air breathing as early as Late Silurian Period (438 million years before present, MYBP), and as recently as Percomorpha in the Cretaceous Period (100 MYBP) (Graham 1997). Air breathing was "invented" at different times, and the factors that selected for this specialization might have been different, therefore, the outcome how air breathing shaped respiratory adaptation in different systematic group of fishes, might be different in extant fishes. For instance, Lepisosteiformes (garfishes) acquired a dual air and water respiration morpho-physiological adaptation in Permian Period (260 MYBP), and Amiiformes (bowfin) in Triassic Period (248 MYBP), whereas some of the Osteoglossiformes (*Arapaima*) in Jurassic Period (213 MYBP). It is both scientifically puzzling and practically important to understand how present environmental conditions of hypoxia and hyperoxia will affect growth performance, morphological, and behavioral responses in these different species.

Garfish experiment (Methods)

Spotted gar broodstock was maintained at Nicholls State University aquatic laboratory and following in-tank spawning, embryos were kept attached to artificial substrate until hatch. Larvae were over-night shipped during the adhesive stage to The Ohio State University. Immediately after the fish arrived in the laboratory, they were placed into tanks at 20.0 °C with approximately 100% oxygen saturation. Fish were kept in these conditions for 5 days until they began freely swimming. When fish reached the free-swimming stage (12 days after hatching), the fish were allocated to 12 tanks consisting of 3 treatments (hypoxia, normoxia, and hyperoxia) and the experiment began (Fig. 1A).

Fish were fed 3 times daily (9:00, 12:00, and 16:00). For the first 30 days of the experiment, fish were fed solely with *Artemia* naupili, and then switched to adult *Artemia.* On the 64th day of the experiment the gar began to be fed with juvenile common carp and fathead minnows. Gars were given live prey fish twice daily.

The experimental system used (a total volume of 532 L) was semi-recirculating with an exchange rate of 1.5 L/minute. Incoming city water was treated within the laboratory by activated charcoal filters and any residual chlorine was removed through addition of sodium thiosulfate to the water. The system consisted of twelve 50 L glass aquaria and a reservoir for heating the water in order to maintain water temperatures at 22.2 ± 1.1 °C. Each tank received a flow rate of 0.3 L/minute. Water recirculating through the system ran through a filtration unit (Aquatic Life Support Filtration Unit, Aquanetics System Pak, San Diego, CA) consisting of biological filter and a filter to remove suspended solids. Photoperiod throughout the experiment was maintained at 13-h light: 11-h dark.

Oxygen was adjusted in each tank separately by using unique in-tank gas columns (Fig. 1). The column was a 25.4 cm piece of 5.1 cm diameter PVC piping. The bottom of the column was capped with 0.5 mm nylon screen, which had a standard air stone at the bottom to inject gas. The gases used to create the three treatments were nitrogen (hypoxia), air (normoxia), and oxygen (hyperoxia). Bio-barrels (Aquatic Ecosystems Apopka, FL) with a diameter of 1" were stacked on top of the air-stone to increase the gas transfer efficiency. At the top of the column, there were holes drilled in the side of the column at the surface of the water for the water that was being pushed upwards by the gas to be expelled. The water leaving the column has the intended level of dissolved oxygen concentration of 40%, 90%, and 180% saturation (Fig. 2).

Sampling and growth measurements
During the first two weeks of the experiment, two fish from each treatment were randomly sampled once a week for histological analysis. After the first 2 weeks, 3-4 fish per tank were sampled on days 21, 53, 63, 71, 104, and 164. Growth data (Fig. 3) were obtained from the weights and length of formalin preserved specimens for histological analysis.

Behavioral observation
Gulping frequency was counted in each tank 2h after feeding. When feeding the fish, fish were fed in five minute intervals to ensure that time since the last meal being fed was equal for each tank. Gulping frequency was measured by counting the total number of times the fish in a tank would return to the surface to release gas and inhale atmospheric air.

Histological measurements
All the gill arches from one side of the fish were separated and each divided into 2-3 sections. In each section, the numbers of filaments were counted and their length measured. Afterwards, a pair of filaments from the middle of each section was separated and the average number of secondary lamellae per 1 millimeter of the filament length determined. The methods for measuring lamellar surface area were essentially the same as those used by Satora and Jakubowski (1995) in a subsequent study. Gills were dehydrated in alcohol, transilluminated in xylene and fixed in Canadian balsam on a microscope slides. The area of 10 well-separated gill lamellae were measured by means of a light microscope with a CDD camera connected to a computer supplied with an analytical program: Image J Wayne Rasband National Inst. of Health, USA. The total surface area GRSA were estimated using methods established by Hughes (1995), and calculated as follows: GRSA = *Lnbl* where *L* is the total length of all gill filaments, *n* is the frequency of secondary lamellae on both sides of the filament, and *bl* is the average bilateral surface area of the secondary lamellae.

The allometric relation between GRSA and body weight was expressed by the equation $Y = aW^b$, or after logarithmic transformation by log Y = log a + b log W, where Y is the parameter analyzed, W is the body mass and log a and log b are parameters of the regression line. After logarithmic transformation data were analysed using linear regression method in STATISTICA 5.0 program (StatSoft Inc., Tulsa, OK, USA). We calculated the both parameters of the regression line, Pearson's coefficient and its statistical significance.

Histological analysis of pseudobranch and swim bladder (day 573)
Following the first phase of the experiment with exposure to different levels of oxygen saturation (day 1-73), recovery in normoxia (day 73-173), fish were maintained in "common garden" single tank design where they could be identified by PIT-tag marking to their original treatment at the early larval/juvenile stage. Fish were fed live fathead minnows, koi carp, and yellow perch and were subjected to the seasonal ambient water temperature changes of 5 to 23^{o}C. For the present studies fish were sacrificed in November 2011, 573 days after hatching. Fish were 20-26 cm in total length.

Three garfish juveniles were sampled for each treatment group, normoxia, hypoxia or hyperoxia, imposed during larval/juvenile stage. Fish were euthanized by immersion in the ice slurry to avoid possible effect of the anesthetic (MS 222). Gills were collected and immersed in either buffered paraformaldehyde solution (4%), Karnovsky fixative, or formalin solution (10%). Pseudobranch (Fig. 7) was dissected out and immersed in paraformaldehyde. Gas bladder was dissected out and cut longitudinally in two pieces, one half was immersed in Karnovsky fixative and the other half in paraformaldehyde. Pseudobranch and gas bladder samples were embedded in methyl methacrylate (brand name). Two gill arches were examined in each case.

Longitudinal histological cross sections (3 mm) of pseudobranch were obtained and stained with toluidine blue (1% alcoholic solution) followed by fuchsin (1% aqueous basic solution) for morphological observations. Neutral mucins were identified by the periodic acid-Schiff method (PAS). Likewise, longitudinal and transversal histological cross sections (3 mm) of gas bladder were subjected to the same staining procedures for morphological and neutral mucins observations.

RESULTS

Sample data from garfish experiment indicate that the range of variation in oxygen saturation had not overlaped between treatments and remained very consistent throughout the study (Fig. 2). This is critical to the conclusion regarding the effect of oxygen saturation on growth of fish as some evidence exists that diurnal oxygen changes may result in compensatory growth rates (Carlson et al. 1980). It was evident that hypoxic condition did not impact growth performance of spotted garfish juveniles (Fig. 3).

We addressed the question of morphological changes in respiratory organs due to environmental oxygen saturation and the only evidence was the increase in filament (total) length in both hypoxia and hyperoxia (Fig. 4). Although a similar trend remained in respect to total respiratory surface area in fish of 173 days old (following acclimation to normoxia), differences were not significant (Fig. 4E).

The frequency of surfacing events that indicate air-breaths (air release and intake) as a function of oxygen saturation was very significant (Fig. 5). Hypoxia in the case of actively feeding fish resulted in high dependence on atmospheric oxygen. An interesting trend was observed following 3 weeks acclimation to normoxia that suggest the fish used to hyperoxia had a tendency to be more relying on gas bladder.

Morphological changes in swim bladder during early ontogeny of garfish indicate an increase in complexity of respiratory surfaces (Fig. 6).

Pseudobranch morphology

Pseudobranch are located under the operculum located specifically in the dorsal part of the inside of each operculum (Kryzanovsky 1934; Fig. 7A). This is the first histological description of this organ in the literature. The pseudobranch presents a single gill arch containing numerous filaments (primary lamella) and secondary lamella. The lamella are well vascularized with a capillary network containing erythrocytes, particularly evident at the tip of lamella. The central cartilaginous tissue is present along the filament axis (Figure 7A, B and C). The epithelium of pseudobranch filaments is formed by variable (2-5 layers) of pavement cells. However, it is not clear if it can be unequivocally associated with fish "historical" exposure to normoxia (Fig. 7C) or hypoxia (Fig. 7D). A thin epithelium covering the secondary lamellae is formed by a single layer of pavement cells, which are lying on a basement membrane supported by pillar cells. Pseudobranch has chloride cells (rounded nuclei and a prominent nucleolus) located along the base of the secondary lamellae, surrounded by pavement epithelial cells (Figure 1D). Mucous cells secreting neutral mucins were identified in pseudobranch. Granulations in the epithelium of pseudobranch filaments reacted intensely to Periodic acid-Schiff (Fig. 8 A-F). PAS method detected mucous cells on the surface of the primary and secondary lamella.

Gas bladder morphology

The gas bladder is a large, bilobed structure and fills in the dorsal portion of fish body cavity (Zaccone et al., 2011). It opens to the alimentary tract through a longitudinal slit on the dorsal side of oesophagous (Jaroszewska and Dabrowski 2008). The wall of the gas bladder is formed of paired trabeculae (Fig. 9A) that subdivides the lateral sections of the organ into a sequence of compartments. This wall is formed of three layers shown in Fig. 9B. The external layer consists in a simple squamous epithelium, middle layer in a connective tissue, and internal layer in a simple respiratory epithelium which is formed of ciliated and goblet cells. PAS method detected mucous cells secreting neutral mucins strongly reactive to PAS in ciliated epithelium of gas bladder (Fig. 10A-D). Parts of the respiratory epithelium are intercalated by mucous cells (Fig. 10E and F). There were no significant differences observed in swim bladder structures among fish with different water oxygen saturation conditions in early life history.

BOWFIN EXPERIMENT

Broodstock bowfin were captured from Winous Point Marsh Conservancy, Sandusky, OH on April 26, 2011. All fish were transported to The Ohio State University where they were treated with hormones. Fish were injected 3 times with Ovaprim at 0.6 ml/kg in 24 hour intervals. The ovulating females began releasing eggs to the tank after the 3rd injection. Fish were then stripped of the remaining eggs and fertilized using the dry method. Males were not spermiating and therefore sperm was added by macerating the testes. Embryos were incubated in rectangular tanks at 14°C. The water source used was city water that was de-chlorinated by use of activated charcoal filters and additionally treated with sodium thiosulfate (4mg/L) to keep chlorine levels below 0.1 mg/L. Water in the tank was gently aerated. Embryos began hatching 5 days after fertilization. Eight days after hatching, fish weighing 0.09g were transferred to 12 aquariums at a density of 30 fish/tank.

The experimental system used was semi-recirculating and consisted of twelve 30 L glass aquaria and a reservoir for heating the water in order to maintain water temperatures at 20.9 ± 0.1°C. The 12 tanks were divided into 3 treatments, hypoxia (3.60±0.33 mg O_2 /L), normoxia (7.15±0.25 mg O_2/L), and hyperoxia (16.19±0.96 mg O_2/L) (Table 1). Dissolved oxygen and temperature were measured twice daily, at 9:00 and 16:00. Fish were kept in these conditions until 52 days post hatch, when they were moved to a single tank for communal rearing.

Water flow rate was maintained at 0.3 L minute. Water recirculating through the system ran through a filtration unit (Aquatic Life Support Filtration Unit, Aquanetics System Pak, San Diego, CA), consisting of biological filter and a filter to remove suspending solids. Photoperiod throughout the experiment was maintained at 13-h light: 11-h dark. Oxygen was adjusted in each tank separately by using unique in-tank gas columns described in the previous section on garfish.

Fish were initially fed with *Artemia salina* nauplii before being weaned to Bio Oregeon's BioVeta starter diet and then to BioVeta fry (42% protein, 16% fat). Fish were fed daily at 9:00 and 16:00. From day 44-52 days post hatch, fish weighing 14.5g ± 1.6 were fed *ad libitum* 4 times daily to quantify growth and feed utilization. Prior to being fed all solid fecal matter was removed from the tank by siphoning and during the last feeding the gas exchange columns were removed and cleaned.

After 52 days post hatch, all fish were communally reared. Fish were transferred to a 400L flow through tank. All fish were implanted with 9mm PIT-tags using a 12 gauge needle for identification. After implanting PIT-tags, fish were treated in 50mg/L oxytetracycline for 1 hour to prevent infections.
Sampling and growth measurements

Tank biomass was measurements were taken intermittently. Weight was measured after days 20, 34, 44, 52, 60, and 91 days post hatch. Biomass was measure by removing all fish from the tank and drying them

slightly with a paper towel before being placed in a bucket of water on a scale. The numbers of fish were counted before being placed back into the tank to calculate mean weights.

Behavioral observation

Air gulping frequency was counted in each tank 2 hours after feeding. When feeding the fish, fish were fed in five minute intervals to ensure that time since the last meal being fed was equal for each tank. Gulping frequency was measured by counting the total number of times the fish in a tank would return to the surface to release gas and inhale atmospheric air.

PIRARUCU EXPERIMENT

The transition from aquatic to air respiration is of great importance in the evolutionary history of fishes and occurred multiple times (Graham, 1994; 1997; Perry et al. 2006). If we concentrate on extant fish species that developed respiration bimodality, (air and water) (Graham 1984 identified over 370 species, occurring among 125 genera in 49 families) and did not lead to terrestriality, these event can be associated with changes in atmospheric oxygen tension (15 to 35% over the 500 MYBP). Pirarucu (*Arapaima gigas*), an Amazonian fish, exhibits two means of oxygen acquisition, aquatic and air in distinct stages of its ontogeny (Gonzalez et al. 2010). In young fish (10-100 g), gills structure is similar to those of others aquatic respiration-dependent fish (Brauner et al. 2004; Da Costa et al. 2006; Ramos 2008). However, an adult pirarucu (over 1,000 g) become obligate air-breathers (Steven and Holeton, 1978). In teleost fish, environmental hypoxia or hyperoxia involve the respiratory neuroreceptors response (Jonz and Nurse, 2006), and behavioral and morphological regulations (Herbert and Wells, 2001). However, it is uncertain if respiratory epithelium (gills and gas bladder) in pirarucu will differ in response to hypoxia or hyperoxia conditions. Therefore, the objectives of this experiment were to determine the respiratory behavior of pirarucu juveniles maintained in normoxia, hypoxia or hyperoxia conditions, and to describe how oxygen stress caused by hypoxia or hyperoxia affects the gill structure.

Experimental procedure

Pirarucu juveniles were shipped from the Amazon (Roraima State) to the Aquaculture Center (Sao Paulo State University). Up to ten fish were kept in three 100 L-tanks with continuously flowing water and constant aeration (5.3±0.2 mg L^{-1} of dissolved oxygen) during two weeks of acclimation. Temperature was maintained at 29.4±0.4 °C using thermostats. The juveniles were fed *ad libitum* with extruded commercial diet for carnivorous fish twice a day (450 g Kg^{-1} crude protein).

Twenty four juveniles (17.3±5.7 g wet weight and 15.0±1.7 cm total length) were distributed to eight 50 L-tanks and two different environmental conditions were established: hypoxia and hyperoxia. Four replicates for each condition were used. Hypoxia and hyperoxia conditions were obtained by injecting either nitrogen or oxygen. One air pump was placed in each tank to ensure uniform gas distribution in the water. Dissolved oxygen concentration was monitored every hour for 18 hours, and its concentration was maintained at 1.3±0.1 mg L^{-1} (17% O_2 saturation) in hypoxia tanks and at 17.9±0.6 mg L^{-1} (240% O_2 saturation) in hyperoxia tanks. Behavioral observations included the frequency of air-gulping. The number of air gulps at the surface was monitored during normoxia, before the onset of hypoxia/hyperoxia exposure. Four juveniles were randomly observed for three minutes over an hour at intervals of 15 minutes. After hypoxia or hyperoxia exposure, one fish per tank was observed, filmed with a digital camera, and the number of times that fish took air at the water surface was recorded. Monitoring took place at the time when fish were transferred to hypoxia/hyperoxia tanks and after 1, 2, 4, 6, 8 and 18 hours of exposure. Observations lasted 3 minutes in all periods. After 18 hours, gas injection was discontinued and normoxia conditions were restored with aeration.

Histological analyses

Three juveniles were sampled for histological analyses before hypoxia or hyperoxia exposure. Then, one juvenile per tank was sampled at 8 and 18 hours of hypoxia or hyperoxia exposure. Fish were sampled again 6 hours after the restoration of normoxia. The juveniles were anesthetized with benzocaine solution (0.1 g L^{-1}) and euthanized by immersion in the ice slurry. Gills and gas bladder were dissected and fixed in 4% buffered paraformaldehyde solution. Samples were embedded in Historesin® (Leica, Germany). Longitudinal histological cross section (3 µm) of gills were cut and stained with 1% alcoholic toluidine blue followed by 1% aqueous basic fuchsin for morphological and morphometric observations. Neutral mucins were identified by the periodic acid-Schiff method (PAS). Acid mucins were identified by Alcian blue pH 2.5 staining technique. Lamella total height (LTH), lamella potential functional height (LPFH), filament epithelium thickness (TEF), lamella epithelium thickness (TEL), interlamellar distance (ID) and lamella width in basal portion (LW) were measured in 20 lamellae per fish in discontinuous views, using an image analysis system (Leica IM50, Germany). Morphometric analysis of gills was based on previous study with pirarucu (Ramos 2008).

Statistical Analysis

The variables were analyzed by two multivariate statistical methods: hierarchical cluster analysis and non-hierarchical and principal components. Analyses were performed using the software Statistica 7.0. To reduce errors due to scales and units of the selected variables, the data were standardized with 0 mean and variance 1. The hierarchical cluster analysis was performed by calculating the Euclidean distance by the method of Ward connection, for all sets of variables (Ward 1963). After identifying the groups formed, analysis was performed by the non-hierarchical or K-means procedures. The principal component analysis allowed for identification of the separation and the interrelationship between the original variables.

RESULTS

Air breathing behavior

In normoxia condition, all fish observed rose once to the water surface for air breathing during the three minutes of monitoring. As soon as the juveniles were transferred to hypoxia and hyperoxia tanks, their air ventilation decreased (Fig. 14). Later, fish maintained in hyperoxia condition showed air ventilation frequency similar to normoxia. In general, hypoxia increased air gulping frequency in juvenile piraruku.

Gills morphology

In normoxic condition, before the onset of hypoxia and hyperoxia test, juvenile pirarucu showed gill filaments well vascularized and the numerous secondary lamellae, which constitute the active respiratory/excretory membrane. Gill filaments have afferent and efferent arterioles, and a central capillary network filled with erythrocytes (Fig. 15A). A central cartilaginous tissue supports the gill filaments. The epithelium of gill filaments is formed mainly by pavement cells, although this epithelium is also rich in chloride cells. Chloride cells (ionocytes) are located along the base of the secondary lamellae, surrounded by pavement epithelial cells (Fig. 16B). Large PAS negative areas were observed in histological sections of the gills (Fig. 16A). A few cells that produce neutral mucins were identified at the distal ends of the lamella.

During hypoxia exposure, an increase of chloride cells size was observed in the secondary lamellae of piraruku juveniles' gills (Fig. 17A and B). In addition, pavement cells, more turgid and irregular in shape, were observed covering the secondary lamellae in fish maintained in hypoxia condition. Granulations in the epithelium of gill filaments reacted intensely to Periodic acid-Schiff. A layer of mucous, PAS positive, cells were detected covering lamellae surface (Fig. 17B). Some mucous cells also constituted filament epithelium, mainly at the distal end (Fig. 17C). When normoxia was restored, a decrease of chloride cells numbers and recovery in size and shape of pavement cells was observed (Fig. 17C). Large

lamellar areas of PAS negative cells were seen in longitudinal sections of gills (Fig. 17D). Few mucous cells reactive to PAS staining were located in filament epithelium.

Discreet morphological changes were observed in the secondary lamellae of fish gills during exposure to hyperoxia and after the reestablishment of normoxia (Fig.18A-C). The gills of fish maintained in these conditions showed lamellae rich in chloride cells, characterized by large cells with rounded nuclei and prominent nucleolus. A thin epithelium covering the secondary lamellae is formed by a single layer of pavement cells which are lying on a basement membrane supported by pillar cells. PAS method detected mucus on the surface of secondary lamella and few mucous cells were located at filaments and lamellae epithelium, primarily in distal portion of the gill filaments (Fig. 18C).

Gills morphometry

The morphometric results of gill elements are shown in Table 1. These data have shown that piraruku exposed to hypoxia during 8 or 18 hours displayed a decrease of total height (LTH) and lamella potential functional height (LPFH), while epithelium lamella thickness (TEL) and lamella width at the basal portion (LW) have increased. Six hours after normoxia restoration, these gill elements recovered and showed characteristic of normoxic condition. Morphometry of gill elements were unchanged in hyperoxia.

Table 1. **Table 1.** Morphometric analyses of piraruku gills at normoxia, and following 8 or 18 hours exposure to hypoxia or hyperoxia conditions. Values represent the means of 20 measurements per fish.

Measurements			Hypoxia			Hyperoxia		
	(μm)	Normoxia	8	18	Recovery	8	18	Recovery
LTH		87.3	52.4	60.1	83.2	75.7	73.2	90.8
	LPFH	60.84	36.4	44.3	64.1	58.8	55.2	72.0
	TEF	27.0	6.4	17.78	21.2	16.5	18.7	21.1
	TEL	4.4	13.8	7.1	4.3	4.6	4.6	4.9
	ID	29.3	36.0	29.8	31.7	34.4	31.2	38.2
	LW	32.1	31.8	34.2	28.2	28.7	31.8	28.1

Lamella total height (LTH), lamella potential function height (LPFH), epithelium filament thickness (TEF), epithelium lamella thickness (TEL), interlamellar distance (ID) and lamella width in basal portion (LW).

The hierarchical cluster analysis classified the data as homogeneous groups. This is represented by a cluster analysis obtained for standardized data matrices. Thus, it was possible to observe the formation of two groups: group 1 (hypoxia and hypoxia at 8 and 18h) and group 2 (normoxia, hypoxia-normoxia, hyperoxia 8h, hyperoxia 18h and hyperoxia-normoxia).

Cluster analysis showed a significant separation between experimental treatments where in group 1, fish from treatments of 8 and 18h hypoxia were grouped. Group 2 was the remaining treatments: normoxia, hypoxia-normoxia, hyperoxia 8h, hyperoxia 18h and hyperoxia-normoxia, as well as which hypoxia-normoxia, and recovery from the hypoxia to normoxia condition of low dissolved oxygen in water for the normal condition (Fig. 19).

According hierarchical cluster analysis method K-means, group 1 had lower values and higher LTH and LPFH TEL and LW. Group 2 presented inverse values, relative to group 1 for variables LTH, LPFH, TEL and LW (Fig. 20). The principal component analysis produced three significant eigenvalues greater than one and was considered in the analysis: the largest eigenvalue are 3.77 (component greater retention of

original variability), 2.59 and 1.00. The three main components together explained more than 90% of the total variance of the data.

The graphical representation and correlation of variables in principal component variables that characterize the more discriminating in the formation of groups 1 and 2 (Fig. 21). In principal component 1 (PC1) the variables LTH and LPFH are located on the left, showing a negative correlation with respect to TEL and LW variables, which are located on the right and show a positive correlation between them and inverse variables LTH and LPFH. We may assume that the variables TEL and LW are the most discriminate characters of group 1 (CP1 highest correlation with the above 0.7 which in turn is the most retains information of the original variables, 47.06%). The other major components showed 32.43% and 12.53% for CP2 and CP3, respectively. At CP2 the variable TEF had a positive value, however negative correlation to the variables of weight and length. CP3 in the determining variable was the ID.

DISCUSSION

Spotted gar and bowfin are facultative air-breathers (Farmer and Jackson 1998; Jaroszewska and Dabrowski 2009). For this reason, it is possible to use either of these species in order to address questions concerning different oxygen conditions (hypoxia, hyperoxia) in the environment, examining adaptation of the gas bladder and gills to effectively exchange gas, and determine the most energetically efficient means of respiratory activity along with associated cost/benefit of each mode of breathing.

In normally aerated water, at 20^0C, gar accounted for 42% of their oxygen metabolism from their gas bladder. Aerial ventilation increased 1150% and was accompanied by an elevation of pulmonary perfusion in hypoxia. It has been shown that gar actually excrete oxygen from their gills in hypoxic water. When severe levels of hypoxia were tested gills were determined to be ineffective as oxygen uptake organs (Burleson et al., 1998). In the experiment with exhaustive swimming activity in hypoxic condition it was determined that the gas bladder takes over the primary role as the air-breathing organ in supporting active metabolism and recovery. Having a gas bladder that can fully support the metabolic scope (Jaroszewska et al. 2010), gar can maintain activity under hypoxic condition that would incapacitate virtually every other temperate climate fish that relies solely on aquatic respiration (Burleson at al., 1998).

In *Amia* exposed to temperatures below 10^oC air breathing becomes negligible, whereas at 32.4^oC, 20-30 breaths per hour were recorded (Horn and Riggs, 1973). At 20^oC, gills contribute to approximately 65% of the total oxygen uptake, but at 30^oC, bowfin becomes an obligate air breather with diminished gill ventilation and aerial breathing satisfies over 70% of oxygen demand (Johansen et al., 1970). Furthermore, aquatic hypoxia significantly increases air breathing frequency from 4.7 to 9.7 breaths per hour at 20-22oC (Hendrick and Jones, 1993). These values are much lower than air-breathing frequency obtained in the current studies in normoxic and hypoxic conditions (Fig. 13). However, the reason for 5-10 fold higher air-breathing frequency in juvenile bowfin in the current studies could have been longer acclimation time to higher water temperatures, fish size as well as postprandial effect. We also added an important aspect to bowfin physiology that is there was no increase in dependence on air-breathing at chronic hypoxia conditions (10 weeks).

Pirarucu as adults is an obligate air-breathing teleost (Stevens and Holeton, 1978). Adult pirarucus of 2-3 kg ventilate their gills 16-24 times per minute and replaces air in their lung every 1-2 min (Stevens and Holeton, 1978). It was evident in the present experiment that juveniles rely much more on gill respiration than air-breathing (see Table 1). In adult fish about 75% of their oxygen consumption comes from air and 63% of carbon dioxide is excreted via gills. Brauner et al. (2004) and Gonzalez et al. (2010) analyzed gill structure in pirarucu in the size range of 10-1,000 g and concluded that the secondary lamellae respiratory surface disappear between 67 and 110 g body weight. However, in none of the studies was the respiratory gas bladder examined during the transition from water to air breathing. Furthermore, the effect of

environmental conditions (oxygen saturation) on the morphological changes and related growth rate (scope for metabolism and activity) was not analyzed in pirarucu ontogeny. Impact of this ontogenetic change from "aquatic" to "terrestrial" respiration mode is critical to aquaculture production capacity and fish growth rate.

Therefore, we conclude that bowfin when maintained at water temperatures of 26-30^{o}C would be an ideal surrogate species for pirarucu in order to study responses of respiratory tissues to changes in environmental conditions. To develop a model species for generalization of physiological responses is certainly a challenging task. However, some aspects of fish response to variable oxygen levels such as respiratory organs morphology and particularly respiratory neuroepithelial cells responses are similar in teleosts and mammals (see reviews by Jonz and Nurse 2006; Jaroszewska and Dabrowski 2010). No studies were performed to our knowledge on the ontogenetic changes in gill and gas bladder morphology in this species despite extensive research done by evolutionary morphologists and embryologists in the 1900s (Ballard, 1984).

CONCLUSIONS

Two temperate fish species, gar and bowfin, showed that gas bladder respiration, manifested by increased rate of air breathing, provides sufficient amount of oxygen resulting in uncompromised growth in hypoxic conditions. Morphological changes in the gills, pseudobranch, and swim bladder during early life history demonstrated enormous plasticity of the species and capacity to acclimation to variable conditions. Importantly, air-breathers are important for aquaculture (Mexico) or have a potential to be cultured for caviar (bowfin).

We observed substantial changes in pirarucu juveniles gill morphology due to short term imposed hypoxia. These changes included presence of multiple layers of interlamellar epithelium and expeditious return to a single layer of pavement, mucus and chloride cells following recovery in normoxia.

All species represent great opportunity for aquaculture because of their unique resistance to variable oxygen conditions, fast growth, and high consumer demand. Our observations and findings are also important in the larger context of climate change along with increasing draught frequency and severity in the tropics (Amazon) and temperate zone. The warming trends will impact fish diversity and although it may prove advantageous to culture of these species, the effects on air-breathing fish populations are hard to predict.

REFERENCES

Burleson, M.L., Shipman, B.N., Smatresk, N.J., 1998: Ventilation and acid-base recovery following exhausting activity in an air-breathing fish. *J. Exp. Biol.* 201: 1359-1368.

Brauner, C.J., Matey, V., Wilson, J.M., Bernier, N.J. Val, A.L. 2004: Transition in organ function during the evolution of air-breathing: insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *J.exp.Biol*., 207: 1433-1438.

Farmer, C.G., Jackson D.C., 1998. Air-breathing during activity in the fishes *Amia calva* and *Lepisosteus oculatus*. J. Exp. Biol., 201, 943-948.

Gonzalez, R.J., Brauner, C.J., Wang, Y.X., Richards, J.G., Patrick, M.L., Xi, W., Matey, V., Val, A.L., 2010: Impact of ontogenetic changes in branchial morphology on gill function in *Arapaima gigas*. *Physiol.Biochem.Zool*., 83: 322-332.

Graham, J.B., 1997: Air-Breathing Fishes, Evolution, Diversity and Adaptation, Academic Press, San Diego, California; pp. 299

Hendrick, M.S., Jones, D.R., 1993: The effects of altered aquatic and aerial respiratory gas concentrations on air-breathing patterns in a primitive fish (*Amia calva*). *J. exp.Biol.,* 181: 81-94.

Horn, M.H., Riggs, C.D., 1973: Effect of temperature and light on the rate of air breathing of the bowfin, *Amia calva*. *Copeia* 1973: 653-657.

Jaroszewska, M., Dabrowski, K., 2008: Morphological analysis of the functional design of the connection between alimentary tract and the gas bladder in air-breathing lepisosteid fish. *Ann. Anat. – Anatomisher Anzeiger,* 190: 383-390.

Jaroszewska M. and Dabrowski, K., 2009: Early ontogeny of Semionotiformes and Amiiformes (Neopterygii: Actinopterygii). In: *Development of Non-Teleost Fish*, Y.W. Kunz, C.A. Luer, B.G. Kapoor [Eds.], *Science Publishers Inc*. pp. 231-275.

Jaroszewska, M., Dabrowski, K., Rodríguez, G., 2010. Development of testis and digestive tract in longnose gar (*Lepisosteus osseus*) at the onset of exogenous feeding of larvae and in juveniles. *Aquaculture Research* 41: 1486-1497.

Jaroszewska, M., Dabrowski, K. 2010b. Does a fish with lungs exists? Morphological and physiological adaptations to aquatic hypoxia and hyperoxia. Kosmos 59: 479-496.

Johansen, K., Hanson, D., Lenfant, C. 1970: Respiration in a prmitive air breater, *Amia calva. Respiration Physiol*. 9: 162-174.

Jonz, M.G., Nurse, C.A. 2006. Ontogenesis of oxygen chemoreception in aquatic vertebrates. Resp.Physiol.Neurobiol. 154: 139-152.

Kryzanovsky S. 1934. Die Pseudobranchie. Zoologischer Anz. 58:171- 238.

Perry, S.F., Wilson, R.J.A., Straus, C., Harris, M.B., Remmers, J.E., 2001. Which came first, the lung or the breath? Comp. Biochem. Physiol. A 129, 37-47.

Potter, G.E., 1927. Respiratory function of the swim bladder in *Lepidosteus*. J. Exp. Zool. 49, 45-67.

Rahn, H., Rahn, K.B., Howell, B.J., Gans, C., Tenney, S.M., 1971. Air breathing of the garfish (*Lepisosteus osseus*). Resp. Physiol. 11, 285-307.

Satora, L., Jakubowski, M. 1995. Morphometry of the gill respiratory area in the perch, *Perca fluviatilis*. Acta Biologica Cracoviensia, Zoologica 37: 11-16.

Satora, L., 1998: Histological and ultrastructural study of the stomach of the air-breathing *Ancistrus multispinnis* (Siluriformes, Teleostei). 1998: *Canadian J. Zoology* 76: 83-86.

Satora, L., Wegner, N.C. 2011. Reexamination of the Byczkowska-Smyk gill surface area data for European teleosts, with new measurements on the pikeperch, *Sander lucioperca*. Rev. Fish Biol. Fisheries 22: 1-9.

Stevens, E.D. and Holeton, G.F., 1978: The partitioning of oxygen uptake from air and from waterby the large obligate air-breathing teleost pirarucu (*Arapaima gigas*). *Can.J.Zool*. 56: 974-976.

Ward, J.H. 1963. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association, 58: 236-244

Zaccone, D., Sengar, M., Lauriano, E.R., Pergolizzi, S., Macri, F., Salpietro, L., Favaloro, A., Satora, L., Dabrowski, K., Zaccone, G., 2011. Morphology and innervations of the teleost physostome swim bladders and their functional evolution in non-teleostean lineages. Acta. Histochem. 113: 578-584.

Acknowledments

Studies on garfish and bowfin were in part supported by the grant from Polish Ministry of Science and Higher Education, No. N-N303-321437 (Warsaw, Poland).

FIGURES

Fig. 1 Picture of the experimental system used during gar and bowfin experiments. Each of the 12 aquariums were equipped with gas-exchange columns to manipulate oxygen level.

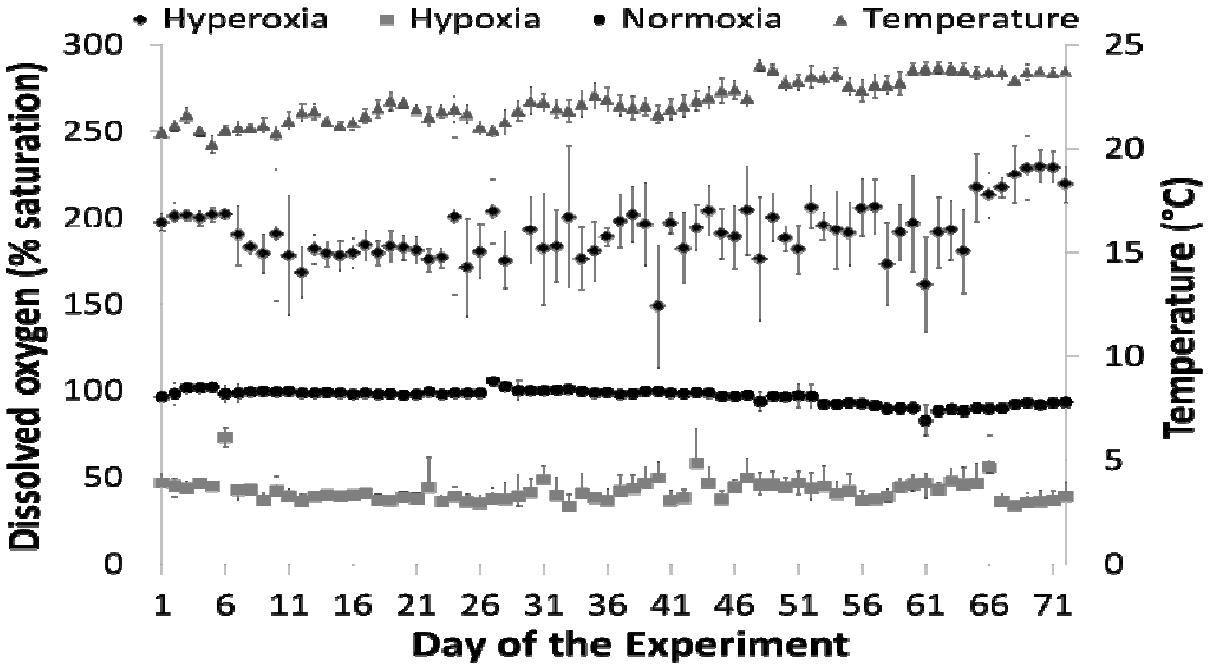


Fig. 2 The daily averages of dissolved oxygen ± standard deviation over the 73 days of oxygen manipulation during the gar experiments. Temperature daily averages ± standard deviations are reported with triangles.

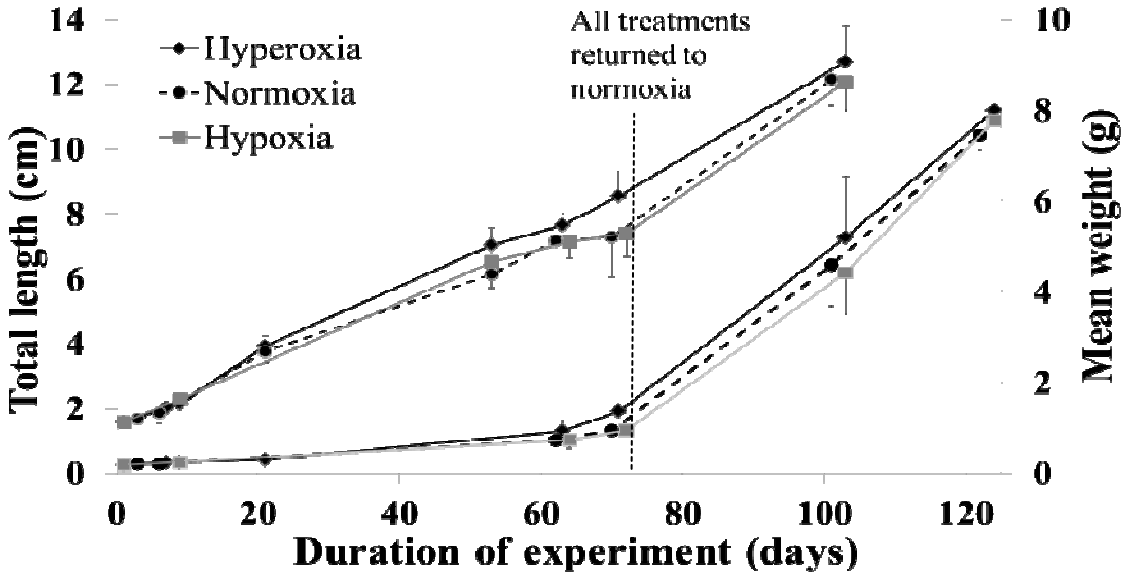


Fig. 3 The growth of the gar throughout both hypoxia and normoxia phases of the experiment. Upper lines represent growth represented in length ± standard deviation, and bottom lines is weight.

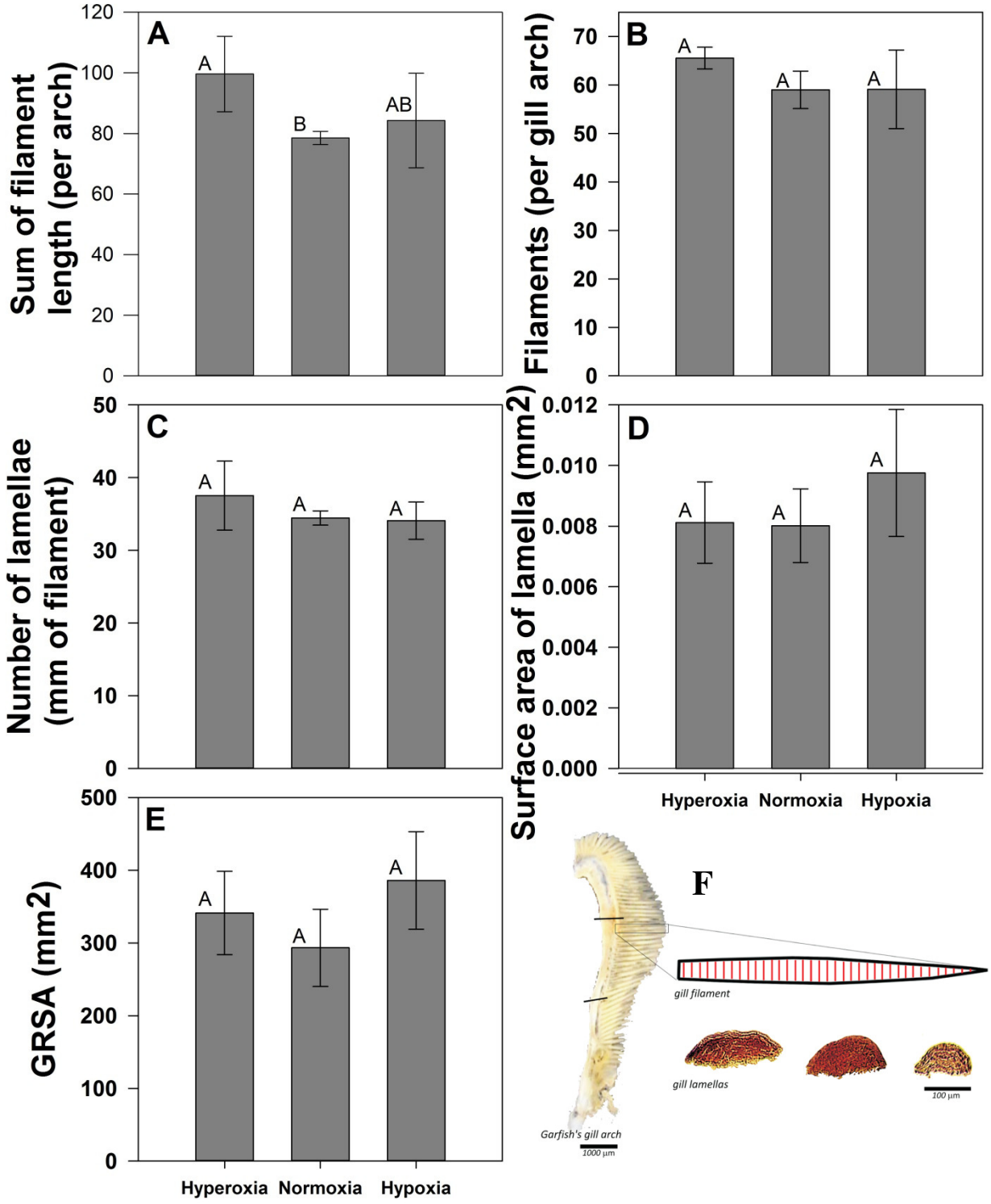


Fig. 4 Mean ± Standard Deviation (n=4). Significance tested using One-way ANOVA Tukey-Kramer method. Graphs A and B are from fish sampled on day 73 of the experiment and graphs C-E are from day 173. F) Schematic drawing to delineate the procedure for measuring gill structure elements.

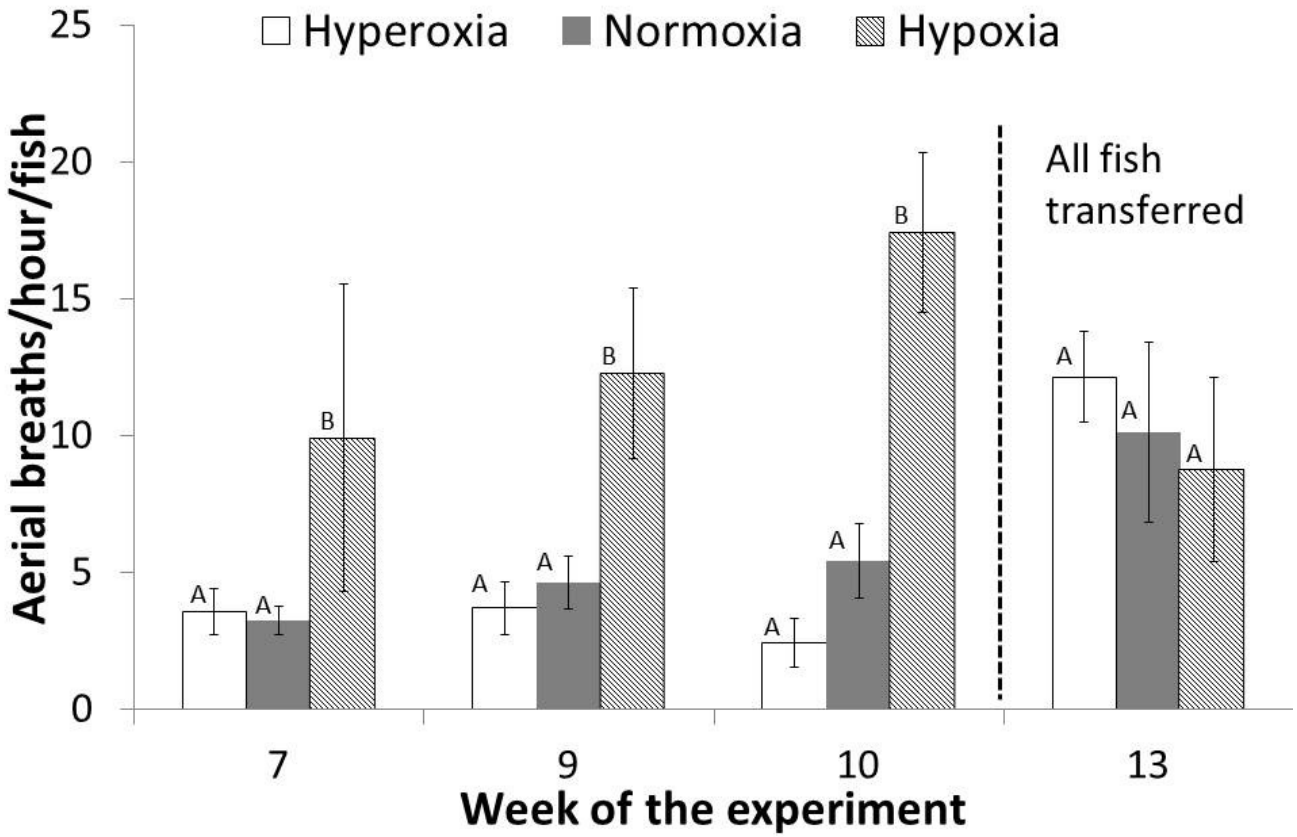


Fig. 5 Mean number of aerial breaths ± standard deviation. Aerial breaths were counted 2 hours after fish were fed. Significance was tested with a One-way ANOVA using a Tukey-Kramer method.

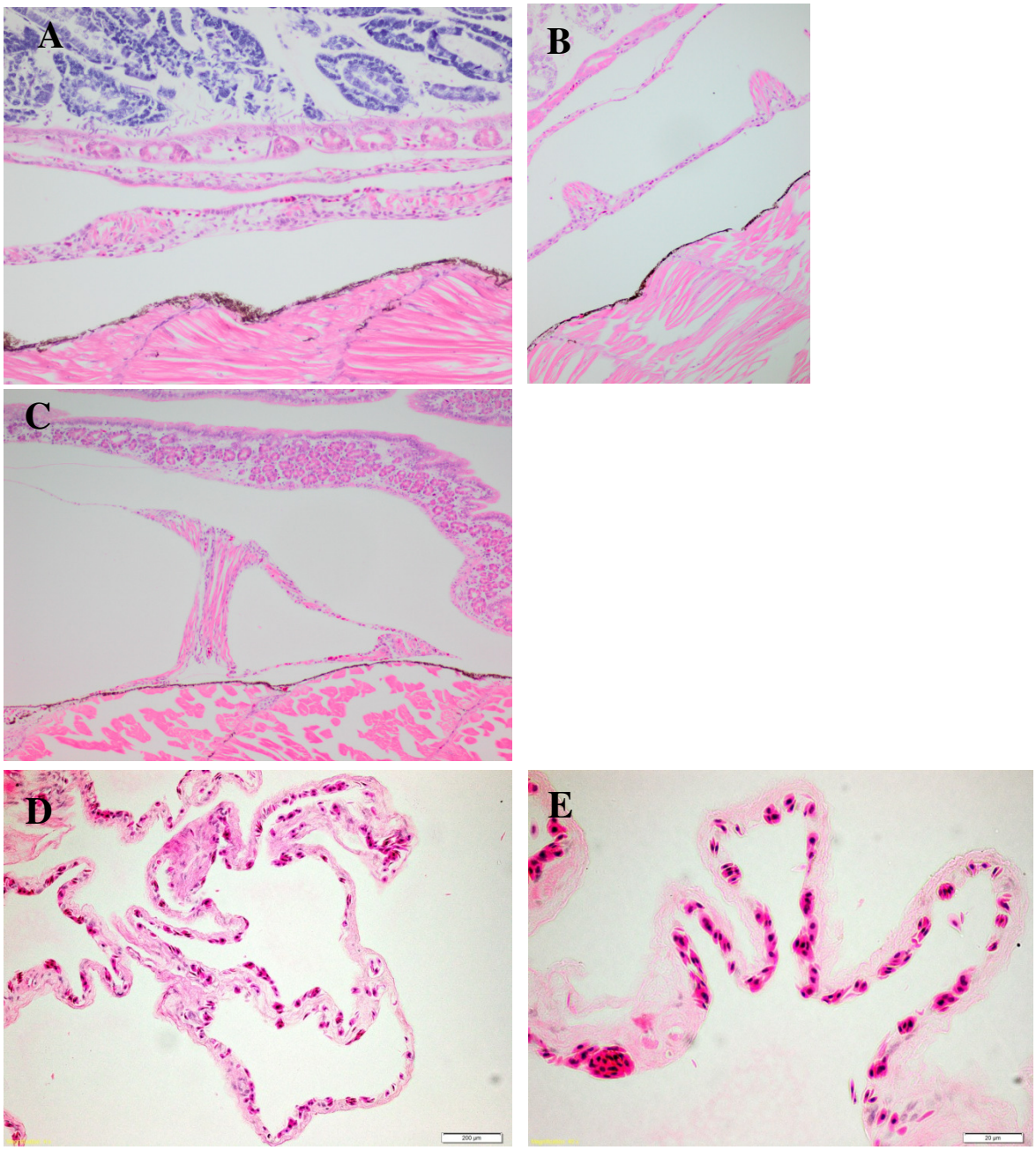


Fig 6. A) Posterior part of swim bladder in garfish 22.3 mm (normoxia, day 7). Note numerous capillaries, particularly on the surface of the ventral part and lack of compartmentalization. B) Fish (23.7 mm, hyperoxia, day 8) with initiation of compartment folds. Striated muscle fibers are present in fold edges. C) Fish (38.8 mm, hypoxia, day 20) with the first compartmental trabecula (*sensu* Potter, 1927) at the tip of posterior part of swim bladder. D) Fish (73 mm, normoxia, day 68) with extensive compartmentalization (swim bladder collapsed during processing). E) Fish (190 mm, normoxia, day 373) with surface of swim bladder folding containing numerous alveolar cells.

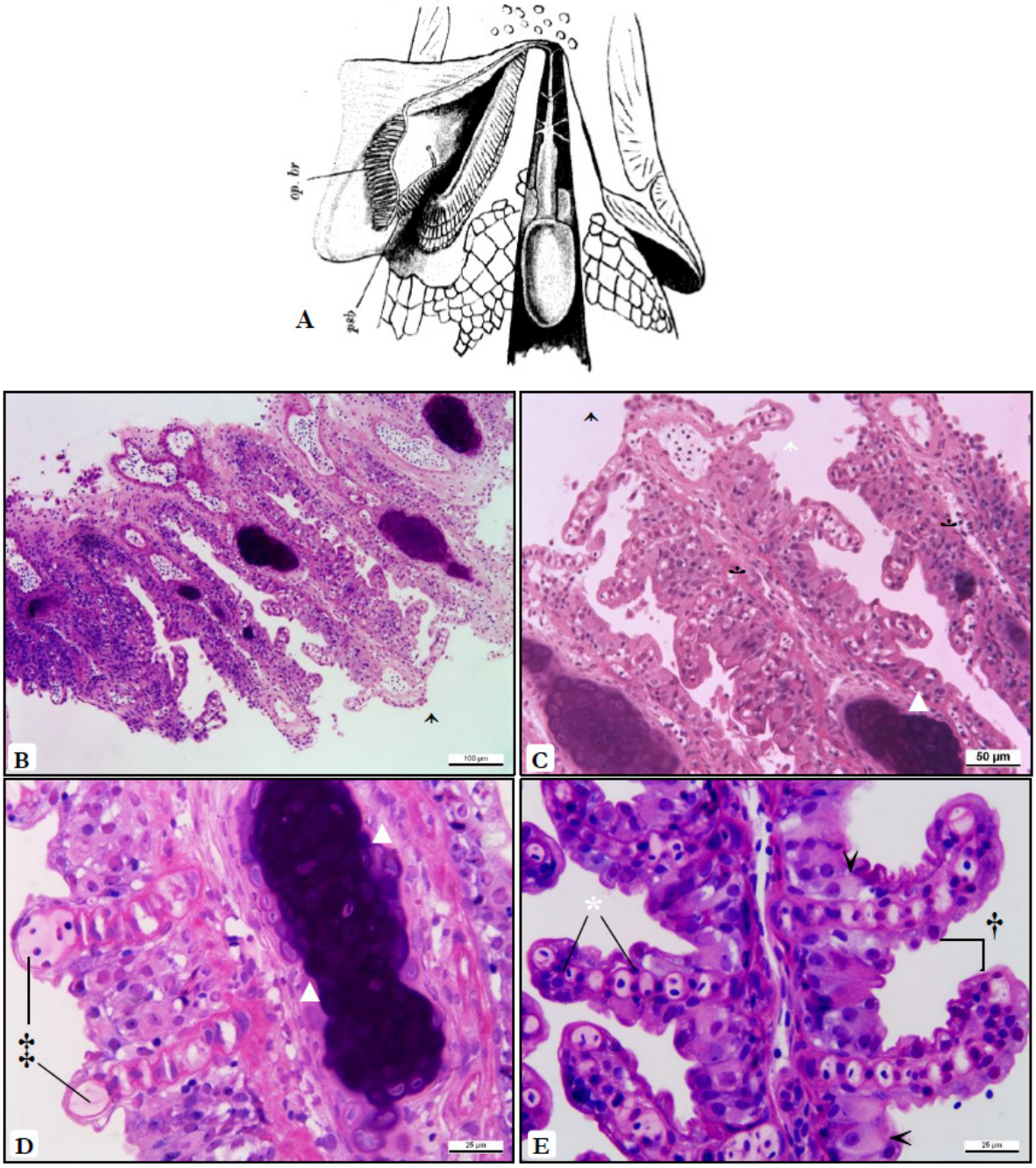


Fig. 7 A) Kryzanovsky S. 1934. Die Pseudobranchie. Zoologischer Anz. 58:171- 238. Longitudinal sections through pseudobranch of spotted gar. Staining method: toluidine blue and basic fuchsin. (B, C, & D) Pseudobranch of fish exposed to normoxia - individual of 20.4 cm; Primary lamellae (filaments) of pseudobranch arch (arrows). Secondary lamellae (white arrows). Erythrocytes within the main blood vessel (asterisks). The lamella displays a central capillary network and erythrocytes (double dagger) within capillary lumen. The pseudobranch filaments are supported by cartilaginous tissue (white arrow heads). (E) Pseudobranch of fish exposed to hypoxia - individual of 21.3 cm; Chloride cells (arrowheads) are usually located along the bases of the secondary lamellae and show rounded nuclei and a prominent nucleolus. Pillar cells (white asterisks). The secondary lamella epithelium is constituted by pavement cells (dagger).

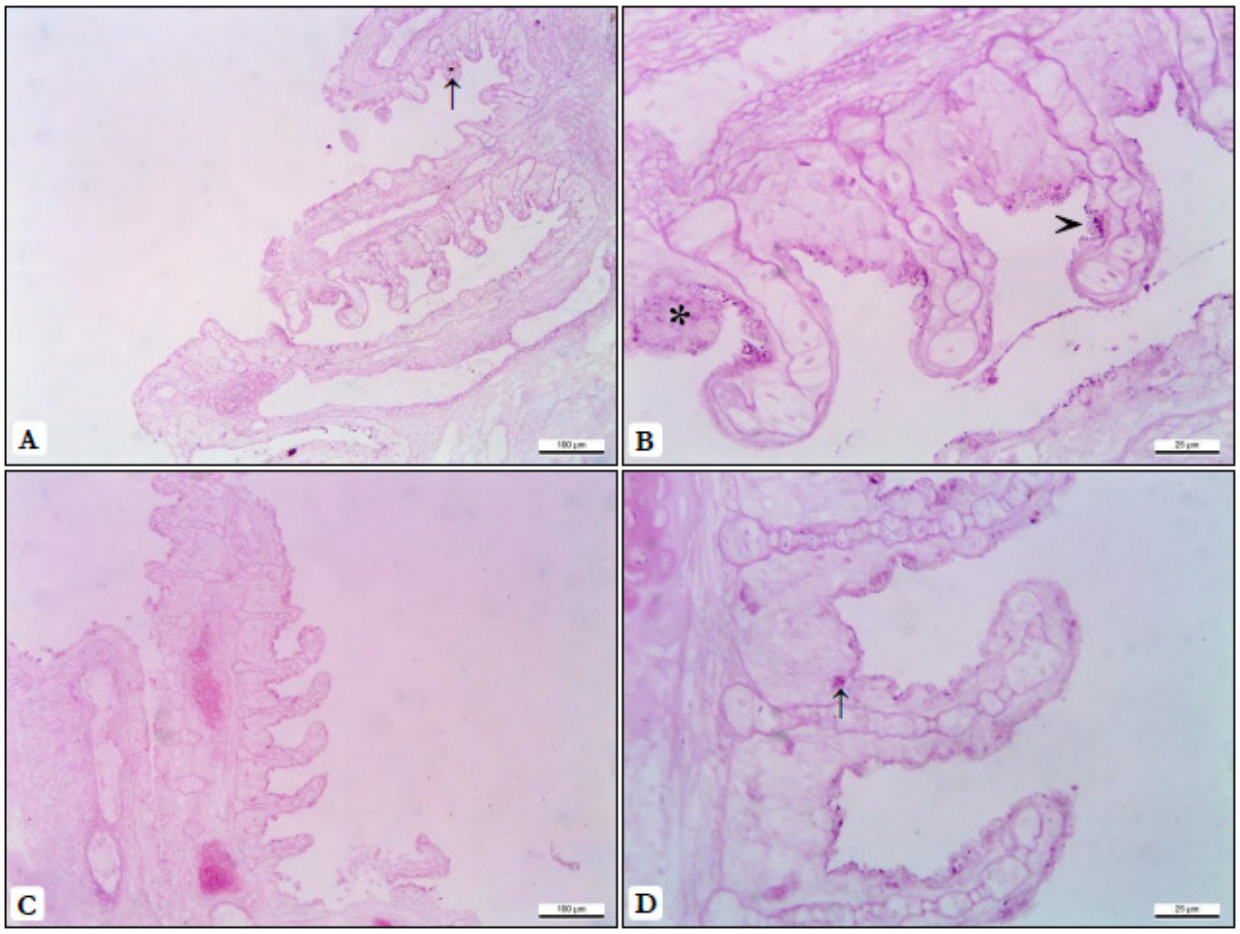


Fig. 8 Histological sections of the pseudobranch of spotted gar (*L. oculatus*) stained with periodic acid-Schiff (PAS). (A and B) Pseudobranch of fish exposed to normoxia - individual of 23 cm. (C and D) Pseudobranch of fish exposed to normoxia – individual of 20.4 cm. Note mucous cells strongly reactive to PAS in filament epithelium (arrows); Granulations PAS+ (asterisks); Mucous covering gill surface (arrowheads).

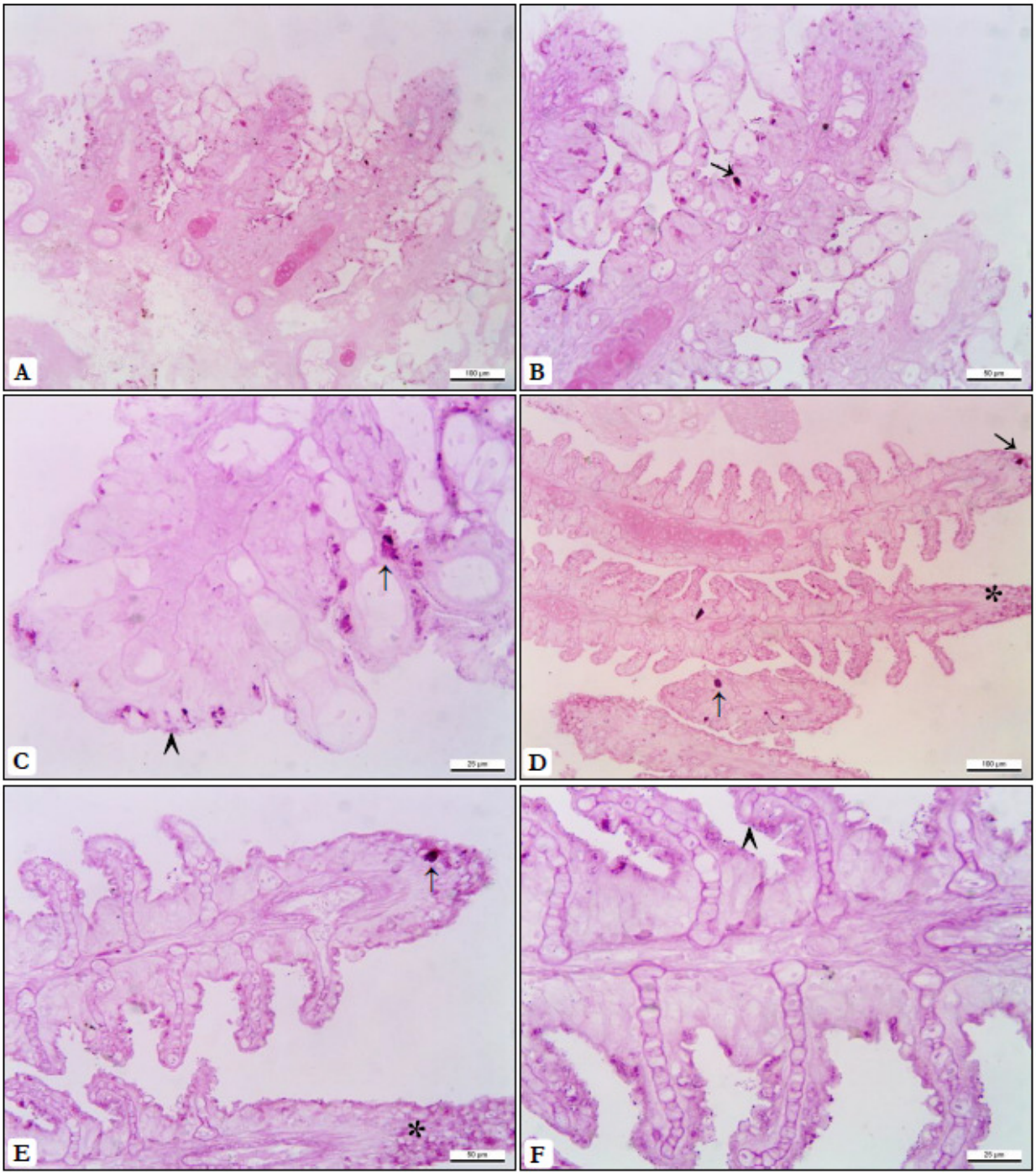


Fig. 9 Histological sections of the pseudobranch of spotted gar (*L. oculatus*) stained with periodic acid-Schiff (PAS). (A, B and C) Pseudobranch of fish exposed to hypoxia - individual of 21.5 cm. (D, E and F) Pseudobranch of fish exposed to hypoxia - individual of 21.3 cm. Note mucous cells strongly reactive to PAS in filament epithelium (arrows); Granulations PAS+ (asterisks); Mucous covering gill surface (arrowheads)

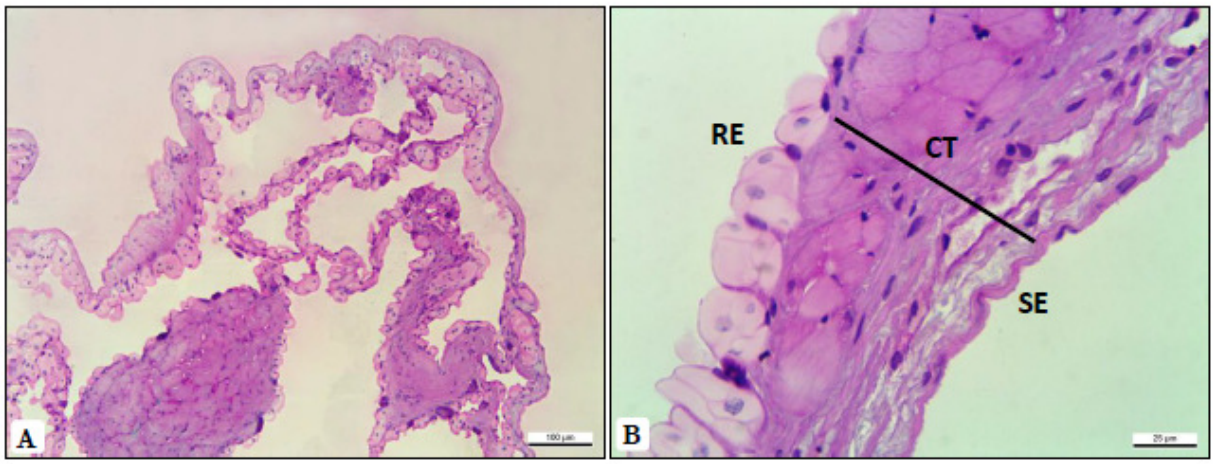


Fig 10. Transversal section through gas bladder of spotted gar juveniles (20-26 cm) Staining method: toluidine blue and basic fuchsin. (A) Trabecular structure. (B) External layer of simple squamous epithelium (SE), middle layer of connective tissue (CT) and internal layer of simple respiratory epithelium (RE).

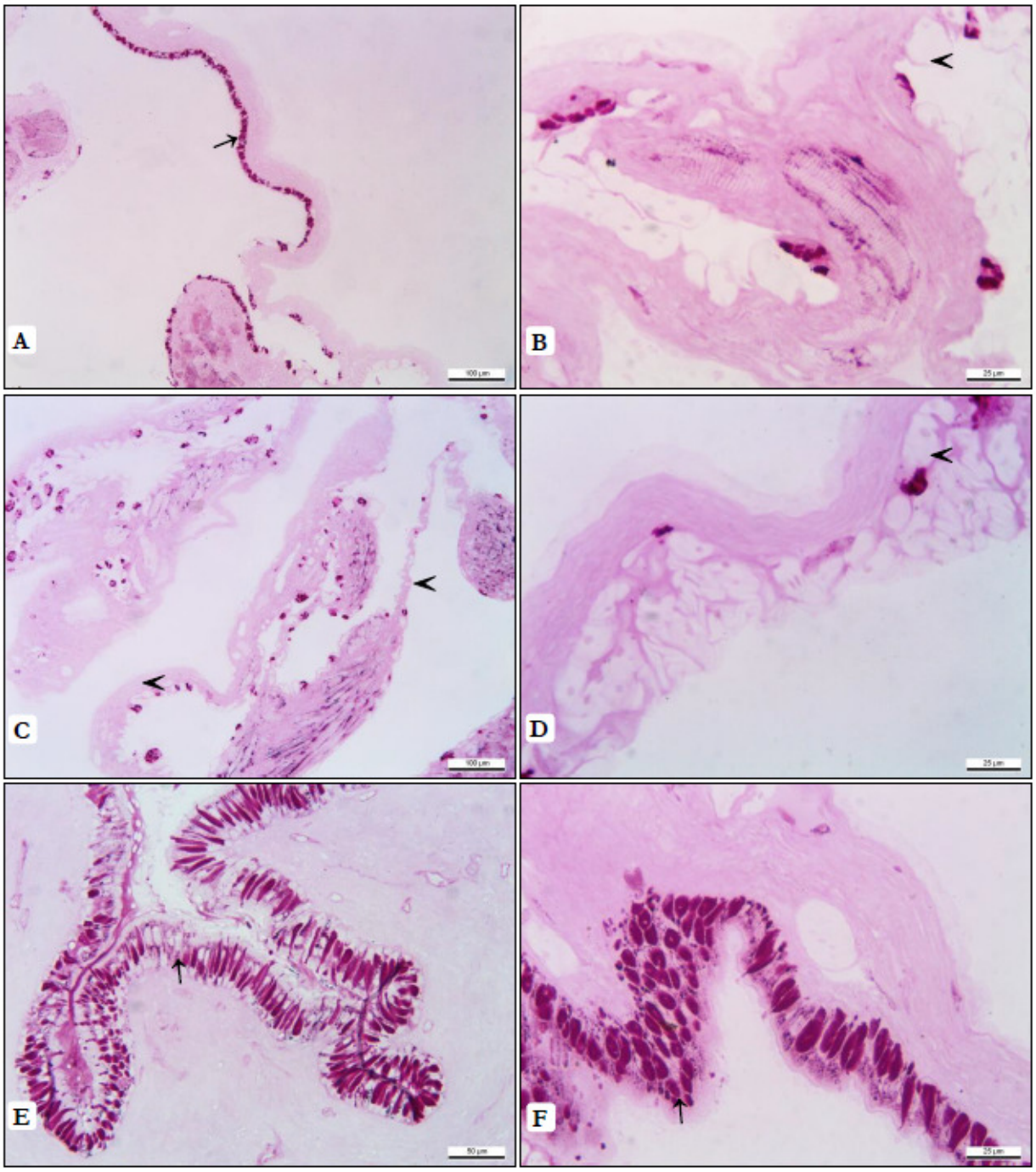


Fig 11. Histological sections of gas bladder of *L. oculatus*.stained with periodic acid-Schiff (PAS). (A and B) Gas bladder of fish exposed to normoxia - individuals of 26 cm and 23 cm, respectively. (C and D) Gas bladder of fish exposed to hyperoxia during early life stages- individuals of 23.2 cm and 20 cm, respectively. Gas bladder of fish exposed to hypoxia - individual of 21.5 cm and 21.3 cm, respectively. Note mucous cells strongly reactive to PAS in ciliated epithelium (arrows); Mucous cells intercalated the epithelium (arrowheads).

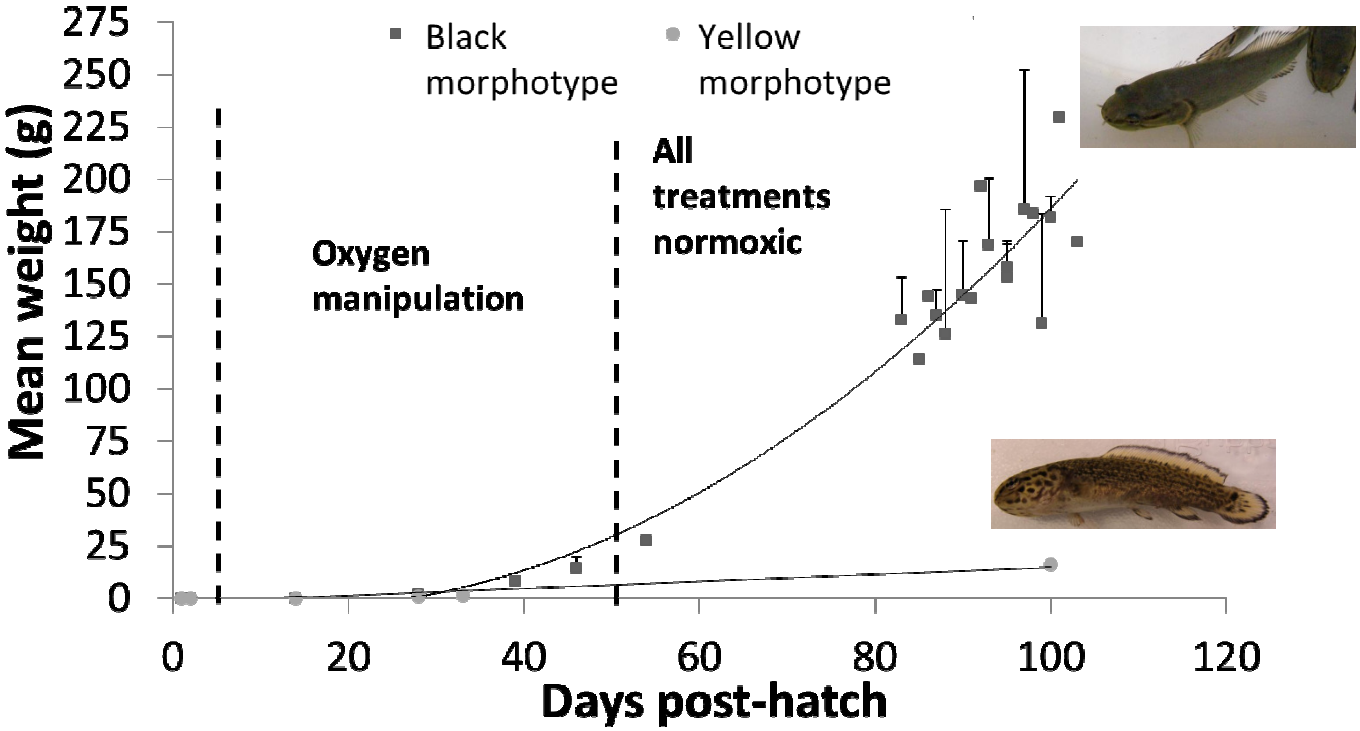


Fig. 12 Comparison of the growth of two morphs of bowfin over100 days. All fish were reared in normoxia throughout the experiment. Growth is expressed as mean weight ± standard deviation.

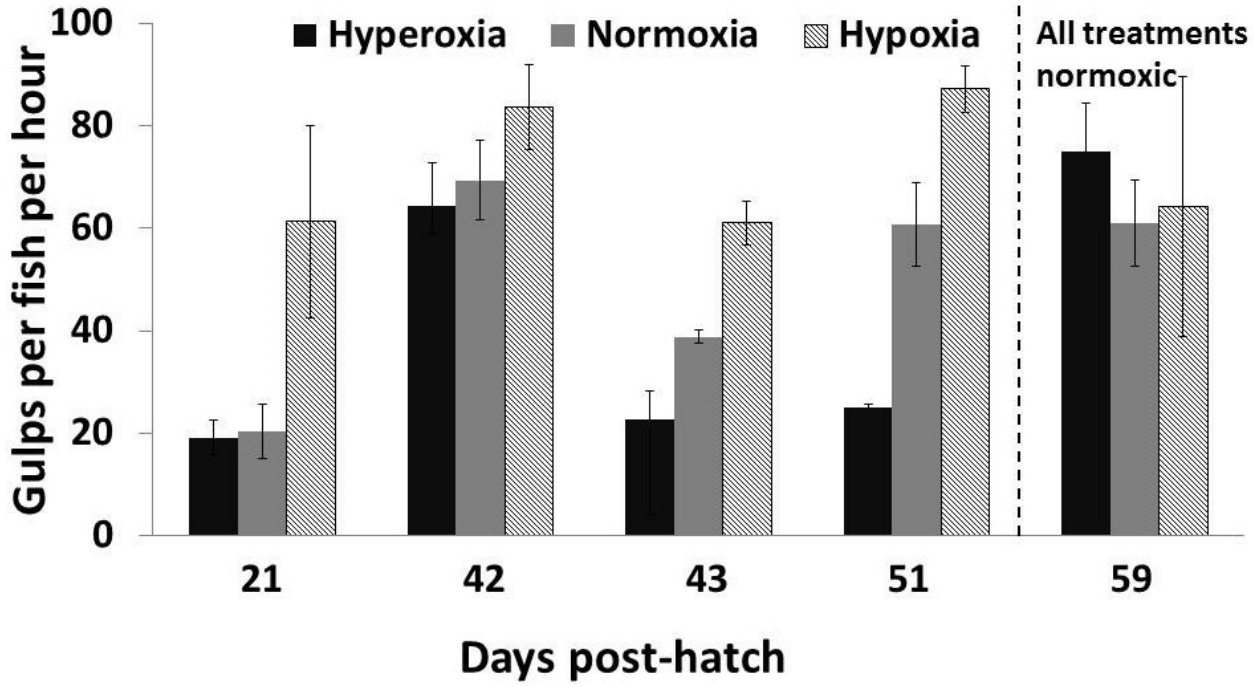


Fig 13. Mean gulp per fish (n=4) ± standard deviation of bowfin during experiment. Gulping was counted 2 hours after feeding for 5 minutes in each tank.

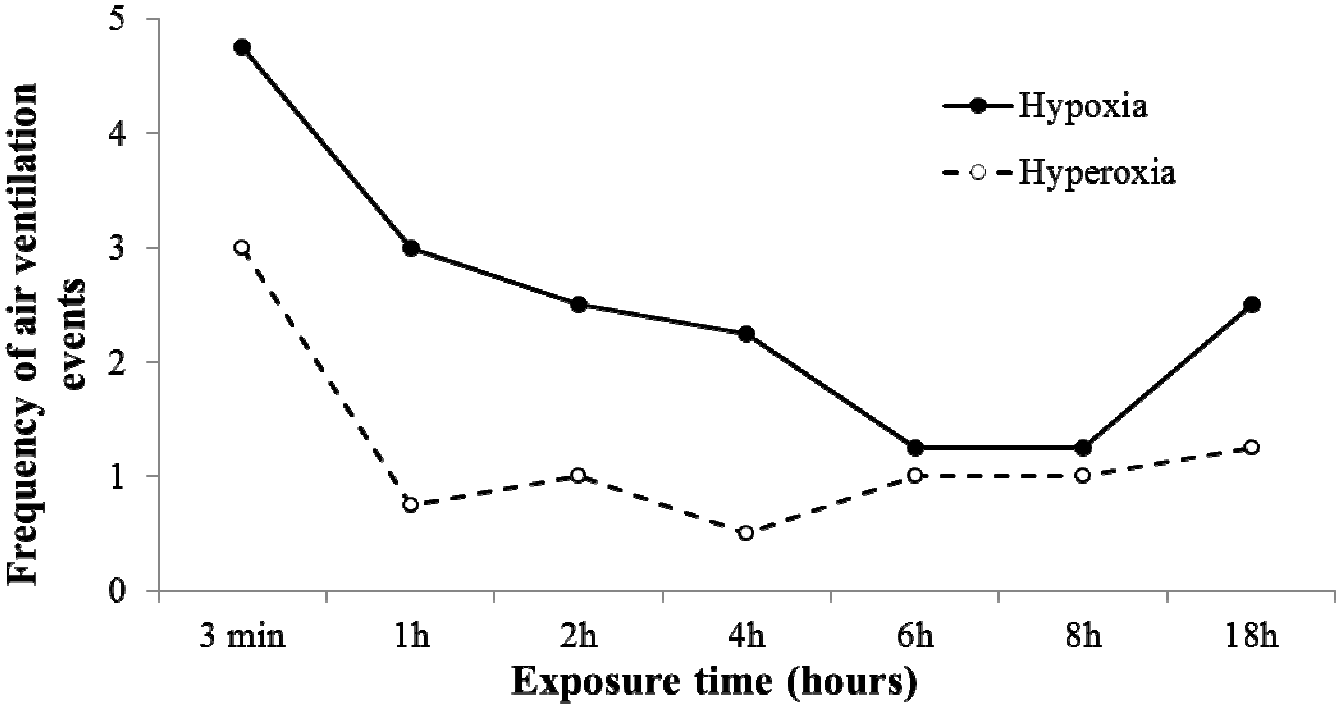


Fig. 14 Number of times that fish return to the surface to for aerial breathing. Data points represent the means (n=4). One fish for each tank was observed for three minutes and number of aerial breathings counted.

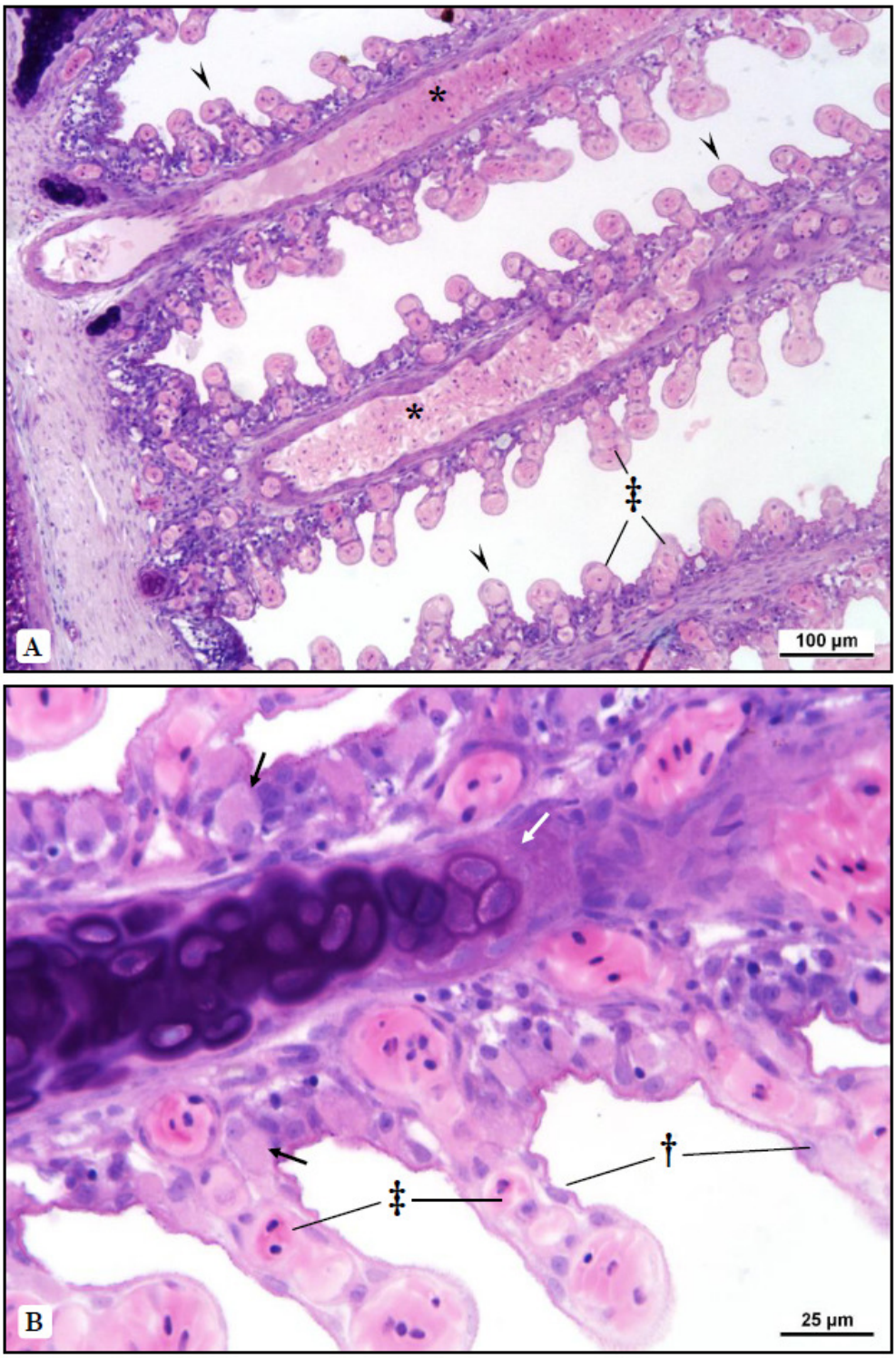


Fig. 15 Transverse sections through gill filaments (or primary lamellae) of *Arapaima gigas* juveniles before the onset of hypoxia or hyperoxia exposure. Staining method: toluidine blue and basic fuchsin. (A) The arrowheads point to secondary lamellae. Erythrocytes are present in the afferent arterial vessels (asterisks) and in the capillaries (double dagger). (B) Stratified epithelium covers the primary lamella. This epithelium is continuous and rich in chloride cells (black arrow) which show round nuclei and a prominent nucleolus. The secondary lamella epithelium is constituted by pavement cells (dagger). This lamella displays a central capillary network and erythrocytes (double dagger) within capillary lumen. White arrow indicates the cartilaginous skeleton, which supports the primary lamella.

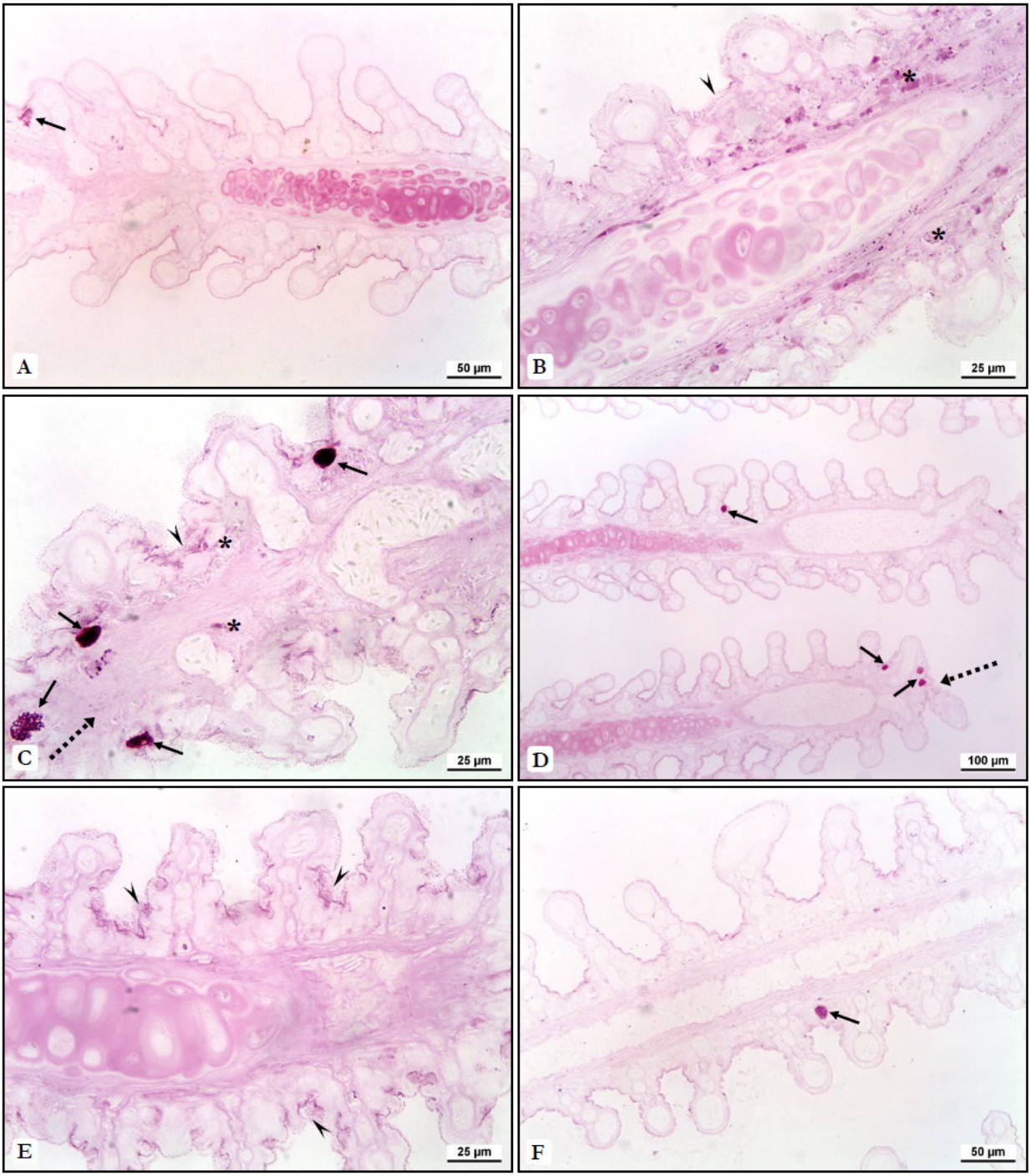


Fig. 16 Histological sections of the lamellar region of *Arapaima gigas* juvenile gills stained with periodic acid-Schiff (PAS). Fish maintained in normoxia, before the onset of hypoxia or hyperoxia exposure (A). Fish exposed to hypoxia conditions for 8 hours (B), 18 hours (C) and restoration of normoxia for 6 hours (D). Fish exposed to hyperoxia conditions for 8 hours (E) and restoration of normoxia for 6 hours (F). Large areas absent in granulations and few mucous cells PAS+ were observed in gill filaments of fish maintained in normoxia condition, including after hypoxia and hyperoxia exposure. Granulations PAS+ located at filament epithelium and a layer of mucous covering gill surface were noted in fish submitted to hypoxia condition. Mucous cells were identified, mainly at distal ends of the filaments (dotted arrow). Note mucous cells strongly reactive to PAS in filament epithelium (arrows). Granulations PAS+ (asterisks). Mucous covering gill surface (arrowheads).

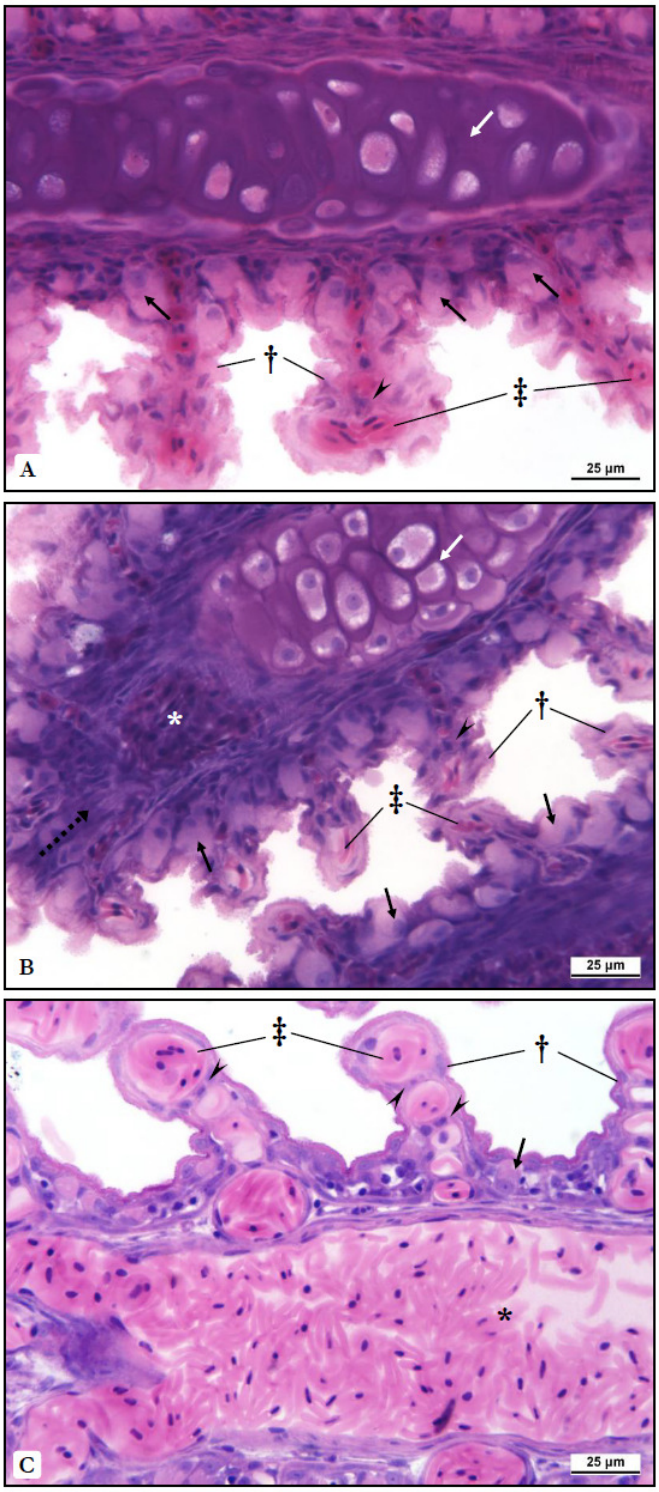


Fig. 17 Histological sections of the lamellar region of *Arapaima gigas* juvenile gills stained with toluidine blue and basic fuchsin. Fish exposed to hypoxia conditions for 8 hours (A), 18 hours (B) and restoration of normoxia for 6 hours (C). See increased size of chloride cells and pavement cells more turgid during hypoxia; after restoration of normoxia, note reduction of them. Blood vessel (asterisks). Central capillary network (double daggers). Pillar cells (arrowheads). Pavement cells, which constitute the respiratory epithelium (daggers). Chloride cells, with rounded nuclei showing a prominent nucleolus (black arrows). Cartilaginous tissue, which supports the primary lamella (white arrows). Epithelium covering the distal ends of primary lamella (dotted arrow).

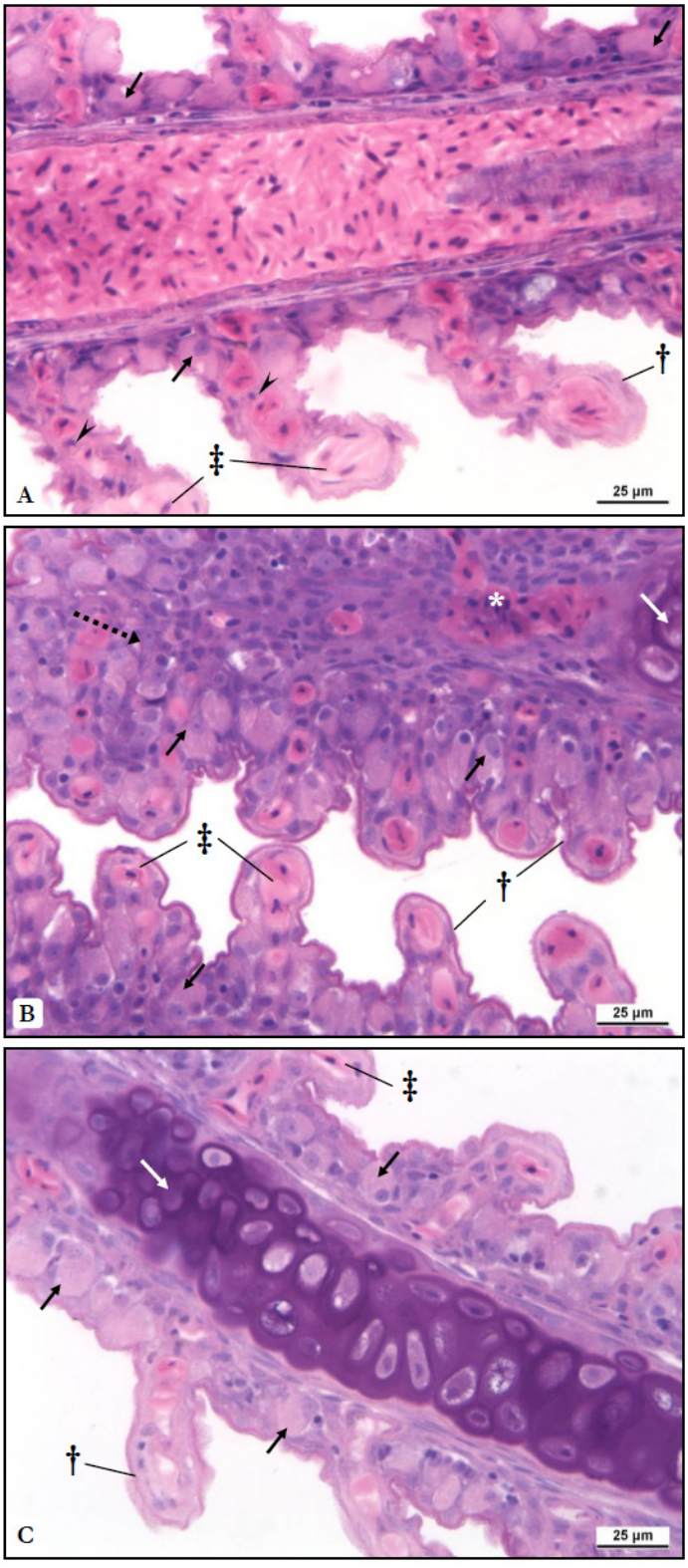


Fig. 18 Histological sections of the lamellar region of *Arapaima gigas* juvenile gills stained with toluidine blue and basic fuchsin. Fish exposed to hyperoxia conditions for 8 hours (A), 18 hours (B) and restoration of normoxia for 6 hours (C). Discreet morphological change was observed in the secondary lamellae of fish gills during exposure to hyperoxia and after the reestablishment of normoxia. Note lamellar epithelium rich in chloride cells (black arrows). Blood vessel (asterisks). Central capillary network (double daggers). Pillar cells (arrowheads). Epithelium pavement cells (daggers). Cartilaginous tissue supporting the primary lamella (white arrows). Epithelium covering the distal ends of primary lamella (dotted arrow).

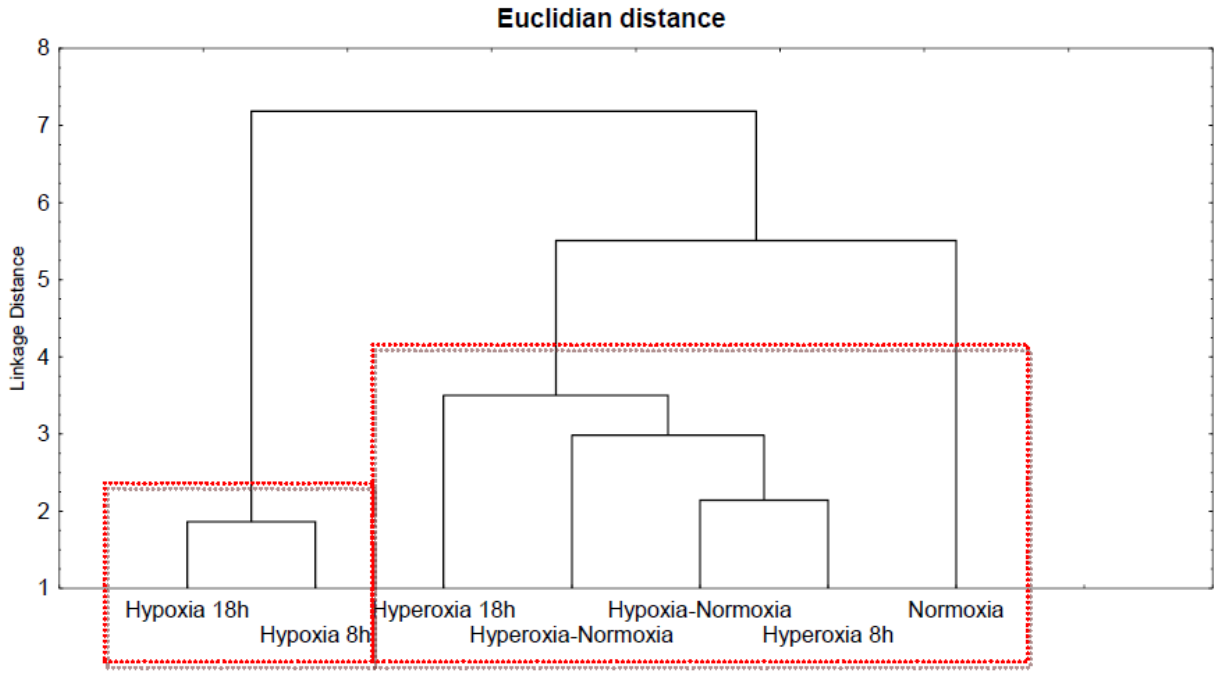


Fig. 19 Dendrogram showing the group structure for the seven variables.

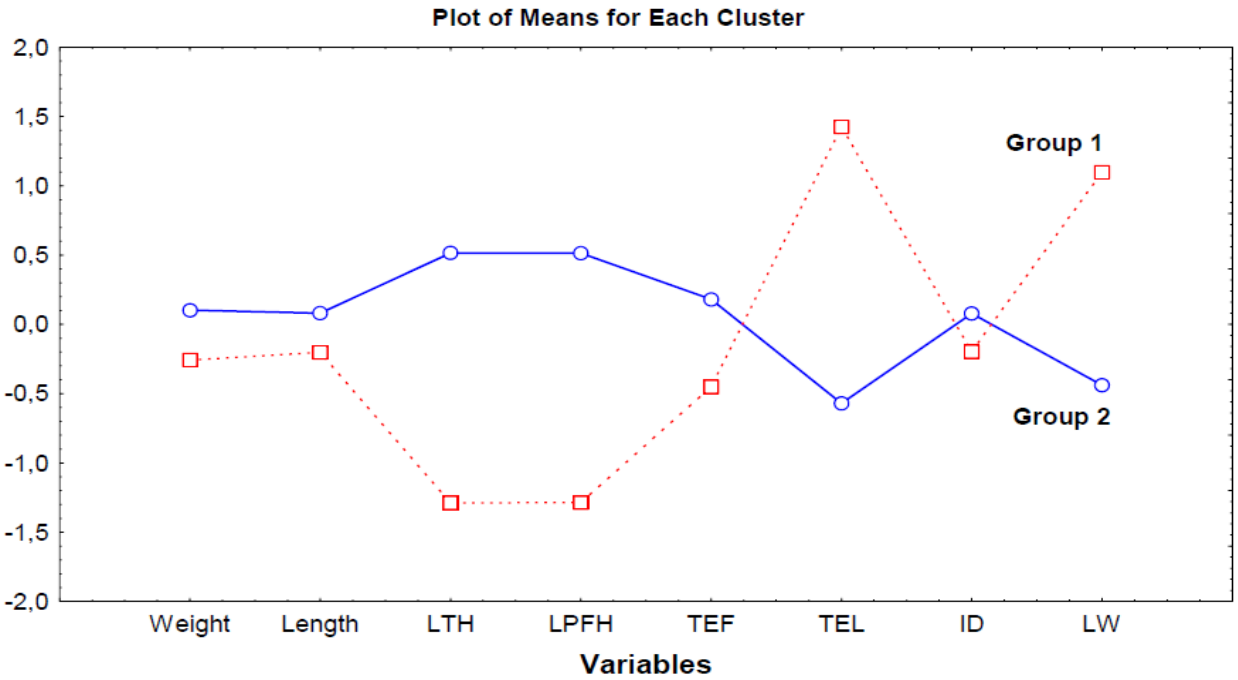


Fig. 20 Grouping by the non-hierarchical or k-means, showing the variables in both groups.

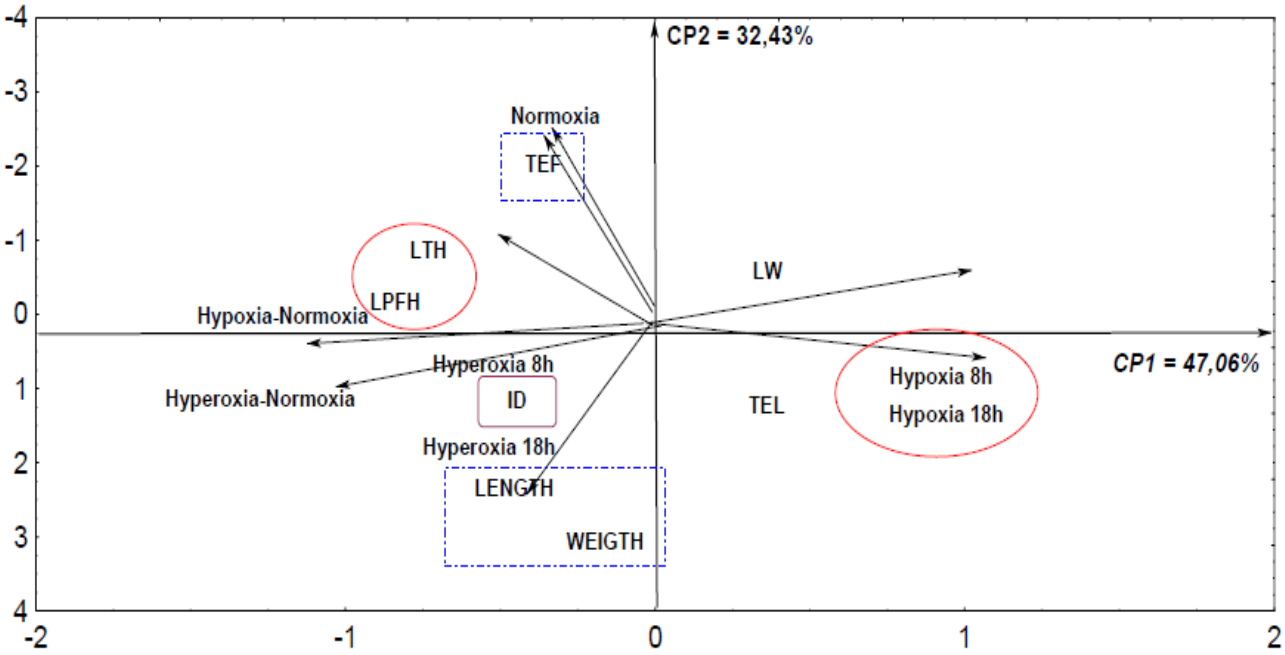


Fig. 21 Distribution of three major components found. Eigenvalues of the principal components: PC1 (47.06%), CP2 (32.43%) and CP3 (12.53%).

Nile Tilapia Broodstock Selection, Seed Quality and Density-Dependent Growth in the Philippines

Quality Seedstock Development/Experiment/09QSD01NC

Emmanuel Vera Cruz and Remedios B. Bolivar
Central Luzon State University
Science City of Muñoz, Nueva Ecija, Philippines

Russell J. Borski
North Carolina State University
Raleigh, North Carolina, USA

ABSTRACT

To support the growing tilapia industry, there is a need to provide year-round, high-quality seed that can be widely distributed at reasonable costs to tilapia farmers. This can be achieved through better broodstock quality, increased hatchery development and enhanced technologies for consistently high-quality seed and fingerling production. Through a series of four studies we assessed if physiological and/or behavioral responses to stress can be used in the selection of broodstock with reproductive advantage in Nile tilapia, examined the effect of broodstock social condition on seed production and fingerling growout performance of tilapia, and evaluated the effects of stocking density on fingerling growth, gene expression of insulin-like growth factor-1 (IGF-1), and stress responsiveness in nursery hapas. In the first study, we investigated whether the outcomes of competition for social dominance among Nile tilapia individuals can be predicted by evaluating the duration of appetite inhibition (DAI) or the feeding response score (FRS) after transfer to isolation. Seventeen fish (70.93%) of the 24 that became dominant have shorter DAI compared to that of their conspecifics (Binomial test, $P = 0.03$). This indicated that social dominance can be predicted using the DAI of the fish during isolation. Reduced growth rate of both dominant and subordinate fish, a well-described physiological end result of social stress, was observed one day after the social interaction. The significantly greater weight loss ($P < 0.01$) in subordinate fish (2.88 ± 0.21 g) compared to dominant fish (2.11 ± 0.19 g) a day after the establishment of social hierarchy was mainly attributed to behavioral differences such as appetite rather than to differences in physical activities. The second phase of this study was similar to the first except we evaluated FRS over a 10-day isolation period as a predictor of social status. Tilapia with higher FRS during the isolation had a higher probability to win the fight for social dominance, indicating dominance can be predicted using the FRS of the fish during isolation, provided that FRS values are not too close to each other. The dominant fish had substantially improved growth that was accompanied by higher expression of hepatic IGF-1 mRNA, a proxy of growth rate ($P < 0.05$). Based on this research, feeding responses of broodstock in isolation are good predictors of social status, such that individuals with low stress responsiveness (higher FRS or lower DAI) are likely to be dominant individuals that can can be selected for as broodstock. In a second study, we assessed the effect of broodfish behavioral stress response on seed production of tilapia through evaluation of their FRS during isolation. Social groups of broodstock representing low stress response (LSR) breeders predicted to be dominant individuals based on elevated FSR during initial period of isolation, or high stress response (HSR) breeders predicted to be subordinate individuals based on low FRS, and their combination were bred in hapas installed in ponds. The greatest number of eggs, largest egg size, highest sperm motility and sperm density, and most number of fingerlings produced were collected in treatments that had both LSR male and female breeders in the group. Higher rates of hatching and survival were also reflected in treatments that had either a LSR male or female in the group. The poorest values of seed quality and quantity were found in breeding groups that contain HSR males and females. These results indicate that stress responsiveness of

broodstock is a good predictor of fecundity and can be used to select fish with higher seed production. Use of feeding responses during an initial period of isolation can be used by hatchery operators and farmers to select broodstock individuals that produce higher quality and quantity of tilapia seed.

In a third, study we evaluated if fingerlings derived from LSR might have improved growth performance relative to fingerlings produced from HSR broodstock during a 3-month pond growout. Sex-reversed fingerlings derived from LSR breeders, had a higher average body weight, a better feed conversion ratio, and an overall higher yield per hectare than fingerlings derived from HSR breeders. There were no differences in survival among the two groups. The results suggests that fingerlings derived from LSR broodstock pose an advantage over those produced from HSR broodstock in overall production performance. This would likely be further amplified were fish growout extended to 4 or 5 months.

An additional study investigated the effect of stocking density on the growth, hepatic IGF-I, and stress responsiveness of fingerling tilapia reared in nursery hapas for 30 days. The overall effect of density as a stressor showed that low density, sex-reversed fish (250 fish/m^3) responded well in terms of growth, specific growth rate, survival, hematological profile (elevated red blood and white blood cell counts) and IGF-1 mRNA gene expressions compared to fish reared at higher densities (500 and 1000 fish/m^3). No clear pattern of difference in blood glucose levels was observed with stocking density. The research demonstrates that density-dependent stress impairs growth through inhibition of IGF-1 production. It is clear that IGF-1 mRNA is a strong growth rate indicator in the field and may also serve as an indirect measure of stress in tilapia. This along with certain hematological parameters may allow for assessment of environmental variables that limit stress and best promote growth in tilapia aquaculture. These data suggest that densities of 250 fish/m^3 are best for growth of fingerlings in nursery hapas.

INTRODUCTION

The quantity of Nile tilapia culture has risen significantly in the Philippines, by almost 4% annually, with a 33% increase between 1997 and 2002 (BFAR 2006 www.bfar.da.gov.ph). To support the growing tilapia industry, there is a need to provide year-round, high-quality seed that can be widely distributed at reasonable costs to tilapia farmers. This can be achieved through better broodstock quality, increased hatchery development and enhanced technologies for consistently high-quality seed production. Here, we aim to assess seed production efficiency in *O. niloticus* as a corollary of broodstock response to social stress. In the aquaculture and breeding environment, fish species such as the tilapia may develop various problems associated with physical, chemical and social stressors (Binurameeh et al. 2005; Chandroo et al. 2004), including impaired reproductive performance. Exposure to stressful conditions can reduce egg size and sperm count, cause ovarian resorption of eggs, delay ovulation, increase developmental abnormalities and reduce size and survival of offspring (Maeda and Tsukumura 2006). The effect of stress on fish is not only determined by the aversive character of the stressor but by the fish's cognitive appraisal of the stressor (Koolhaas et al. 1999). In order to optimize reproductive performance of valuable broodstock and improve seed production, stress must be limited and fish selected for based on their ability to better cope with stress. Breeding is largely driven by social behavior and an understanding or ability to predict dominance, or select breeders with reduced stress responsiveness, can improve hatchery and breeding programs.

The physiological and behavioral responses to stress may be used to select broodstock with reproductive advantage. The variable color pattern in fish may signal a behavioral strategy, enhance camouflage, improve communication, and confer reproductive advantage (Korzan et al. 2008). In *O. niloticus*, eye color is associated with social status (Volpato et al. 2003; Vera Cruz and Brown 2007). In our previous studies, eye color was found to be a predictor and consequence of social rank (Vera Cruz et al. 2009). However, no study to date has examined if eye color pattern is related to behavioral stress responses in *O. niloticus*; for instance, that associated with appetite inhibition or feeding response during isolation.

Moreover, to our knowledge, no study has been done in tilapia to examine if reproductive performance and seed quality is affected by broodstock social rank and/or condition.

To seek higher incomes, farmers in the Philippines are increasingly rearing fish at higher stocking densities in cages, concrete tanks and ponds, including more intensive fingerling production in nursery hapas. Filipino farmers are interested in knowing the stocking densities that yield the best growth rate and minimize mortalities under more intensive culture conditions. Some have noticed significant mortalities in cages, likely due to overcrowding. It is possible that the farmers are stressing, and therefore, reducing the growth potential of fish at higher densities. At lower densities behavioral or social hierarchies may dominate and limit growth potential. Therefore, an additional aim of this investigation will test the effects of stocking density on the growth, survival, and hepatic gene expression of IGF-I, a proxy for growth in *O. niloticus* and other fishes (Picha et al. 2008a), and on stress responsiveness in tilapia. Stress could be measured by a number of factors including survival, growth and elements of the growth axis (i.e. IGF-1), hematological variables (red and white blood cells), glucose, cortisol that is a key hormone mediating stress, and tissue indices (hepatosomatic and cholecystic index) (Bonga et al. 1997).

Physical and social stressors can evoke non-specific physiological responses in fish (Barton 2002). These responses are considered adaptive to enable the fish to cope with the stressful condition and maintain its homeostatic state. If the stressor is severe or long-lasting and the fish is not capable of regaining homeostasis, then the responses themselves may become maladaptive and threaten the fish's health and well being (Barton 2002). Physiological responses to stress can be grouped as primary, which include endocrine changes such as measurable levels of IGF-I (Vera Cruz and Brown 2007) and circulating catecholamines and cortisol (Barcellos et al. 1999) and secondary, which includes changes in features related to metabolism, immune function, and energy depletion reflective in lower liver weights ((Binuramesh et al. 2005; Picha et al. 2006; Picha et al. 2008b). Stressful condition was found to significantly increase circulating cortisol levels (Bolasina et al. 2006), hepatic phosphoenolpyruvate carboxykinase activity (Dibattista et al. 2006), and bile retention (Earley et al. 2004), but it significantly decreases hepatic IGF-I levels (Vera Cruz and Brown 2007). A well-characterized physiological consequence of social stress and excessively high densities is a reduced growth rate (Sloman et al. 2000). Excessively high stocking density is a stressful condition and decreases fish growth (Björnsson 1994), increases plasma cortisol levels in flounder (Bolasina et al. 2006) and decreases survival in salmonids (Sodebergg and Meade 1987). Social stress and the formation of feeding hierarchies, are also density dependent (Vera Cruz et al. 2006). Differential alterations in growth rate between dominants and subordinates are attributed more to behavioral changes (*i.e.* feeding) as transduced by physiological regulators (*i.e.* IGF-I level) but may also be due to changes in metabolism (*i.e.* hepatic phosphoenolpyruvate carboxykinase activity and bile retention) (Earley et al. 2004; Dibattista et al. 2006; Vera Cruz and Brown 2007). The growth-promoting actions of growth hormone (GH) are mediated through induction of IGF-1 (Degger et al. 2000; Picha et al. 2008a). Subordinate or stressed fish is characterized by larger somatostatin-containing neurons in the hypothalamus, which leads to reduced production of pituitary GH (Hofmann and Fernald 2000). Due to this, subordination depresses hepatic IGF-I levels while dominance stimulates its production, likely through greater secretion of pituitary growth hormone (Vera Cruz and Brown 2007). Here, we aim to assess densities that yield good growth and survival while simultaneously evaluating the use of IGF-I and various other factors as markers of growth and stress. An assessment of hepatic IGF-I mRNA in these studies will further validate its usage as a field indicator of growth status in tilapia (Vera Cruz et al., 2006; see Picha et al. 2008a) as well as its putative use as an indirect marker of stress. Additionally, blood glucose, red and white blood cell counts, and tissue indices (hepatosomatic and cholecystic) that can be readily measured will be evaluated as potential indicators of stress that could prove useful tools to evaluate poor environmental conditions. Building biotechnology capacity in the Philippines and development of potential bioindicators can expedite the evaluation of environmental parameters that best promote growth (or limit stress) in tilapia.

OBJECTIVES

1. Determine if physiological and/or behavioral responses to stress can be used in the selection of broodstock with reproductive advantage in Nile tilapia (*Oreochromis niloticus*).
2. Examine the effect of broodstock social condition on seed production and fingerling growout performance of tilapia.
3. Investigate the effect of stocking density on the growth, gene expression of hepatic insulin-like growth factor-I (IGF-I), and stress responsiveness of tilapia.

MATERIALS AND METHODS

Study 1 - The influence of duration of behavioral stress response on social dominance in tilapia

This study aims to investigate whether the outcomes of competition for social dominance among *O. niloticus* individuals can be predicted using the feeding response score and/or duration of appetite inhibition as a stress coping style. In addition, it also investigates if eye color pattern (ECP) is related to the duration of behavioral stress response such as appetite inhibition. The concept is to enable the selection of those broodstock that show dominance and hence might convey reproductive advantages. Physical and behavioral markers such as eye color pattern (ECP) and appetite, respectively, are features that could be easily assessed by hatchery operators in selecting the best mating pairs for seed production.

Phase 1 - Isolation and monitoring of the duration of appetite inhibition (DAI)

This phase of the study was done to assess if the outcomes of competition for social dominance might be predicted by DAI as a stress coping style.

Experimental fish

One hundred size #20 genetically male Nile tilapia *(Oreochromis niloticus),* with average weight of 0.60 g, were obtained from the Phil-Fishgen, Central Luzon State University, Science City of Muñoz, Philippines. They were maintained in a rectangular tank (2m x 1m x 1m) receiving continuous flow of water. The fish were fed three times a day at 3% of the body weight. Prior to isolation weight of each fish was determined.

Isolation and monitoring of the DAI

Fifty fish (mean weight of 26.02 ±0.98 g) were isolated at random in glass aquarium (30cm x 15cm x 30cm) for 10 days. Each isolation unit was aerated to ensure sufficient dissolved oxygen available for the fish. Three sides of the aquarium were covered to prevent the fish seeing other fish isolated in the nearby aquaria. Upon introduction of each fish in the isolation unit, it was immediately hand fed with three pieces of floating feeds placed in a feeding ring. The duration from the time of feed introduction to the time of feed consumption was regarded as the DAI. The DAI and the weight of the fish served as the bases for pairing the fish for social interaction; fish with shorter DAI against those with longer DAI; with both fish having similar weight. Fish were then fed daily at 1% of the body weight except two days prior to interaction. Water exchange was done every other day to maintain good water quality.

Social Interaction

After establishing the competing fish for social interaction, each fish in a pair was individually marked by a small cut on the upper or lower part of the tail fin for the purpose of identifying the fish with shorter and longer DAI. The fish in a pair with longer DAI was cut on the lower portion of the caudal fin and vice versa. After marking the fish, the pair was introduced into a new environment (30cm x 15cm x 30cm aquarium) at the same time to prevent the effect of place familiarity. The period from time of introduction to the time of first agonistic attack was recorded. The number of attacks in a ten minute-time from the first agonistic attack was separately recorded from the total number of attacks during the entire

interaction. Change in eye color pattern (ECP) of the competing fish was monitored at the start, during and after the competitive social interaction. Eye color was quantified as darken area of both iris and sclera (Volpato et al., 2003). The circular area of the eye was divided into eight equal parts using four imaginary lines (Figure 1). Eye color pattern value ranged from zero (no darkening) to eight (total darkening) . At the end of the interaction, social rank (dominant or subordinate) was identified by the characteristics displayed by each fish such as proactive and reactive, pursuing and retreating, erected and not erected dorsal fin and as well as changes in skin color and ECP. Canon power shot A650IS image stabilizer AIAF digital camera with resolution of 12.1 megapixels was used to document the social interaction which in turn was used in checking the observations made during the interaction.

Growth Rate Observation
Paired fish after the interaction were transferred to the dominant fish's aquarium to support its dominancy status. They were maintained for a week and fed once a day at 3% of their total body weight. Exchange of water was done every other day to maintain good water quality. The weight of fish was recorded a day after the fight.

Statistical Analyses
Frequency difference was analyzed using Binomial test. Mean DAIs of the two groups, and mean decrease in weight a day after the social encounter between dominant and subordinate fish were compared using paired sample T-test. Linear relationship of DAI and aggression was assessed using linear regression and Pearson correlation coefficient. Statistical analyses were carried out by using the SPSS software version 16.0.

Phase 2 - Feeding latency as an indicator of stress coping style
The second phase followed similar protocol as the first phase except we evaluated whether the outcomes of competition for social dominance among *O. niloticus* individuals can be predicted using the feeding latency score as a stress coping style. The feeding latency scores allow a quantitative evaluation of the feeding response exhibited throughout the whole isolation period.

Experimental Fish
The Freshwater Aquaculture Center (FAC) Selected Tilapia (FaST) strain of Nile tilapia fingerlings were obtained from the FaST project, FAC, CLSU, Science City of Muñoz, Nueva Ecija. Three hundred mixed sex tilapia of same age and similar sizes were reared in a rectangular tank (2 x 1 x 1 m) and fed two times a day at 3% body weight. Upon reaching 10 grams, male fish were separated from the females.

Feeding Latency During Isolation
After rearing in the rectangular tank, the fish were individually weighed and then isolated for 10 days in 30 x 30 x 15 cm aquarium. The fish were fed once a day at 1% of the body weight except during the last 2 days of the isolation period. The time from introduction to a new environment to the first acceptance of food was monitored for each of the fish. Feeding behavior during the entire isolation period was quantified by assigning corresponding point scores for a particular feeding behavior as shown below:

Point	Behavior
0	Fish does not react or eat the food
1	Fish eats only pellets directly put in front of it, and does not move to eat the food
2	Fish moves to eat the food, but comes back to its original place in the aquarium between each feeding
3	Fish eats all food present in the aquarium

After the isolation period, the daily scores were summed up to get the feeding response or latency score (FRS). Water exchange (50%) was done every other day to maintain good water quality. Water temperature was monitored daily at 7 AM and 2 PM.

Eye color was monitored daily during the isolation period. Eye darkening has been studied in other fish using gradual color patterns transformed into scores. Eye color was quantified as darkened area of both the iris and sclera as described above. Eye color pattern value ranged from zero (no darkening) to eight (total darkening).

Social Interaction

Fish with the same sex and similar weight but with different FRS were then subjected to social interaction. A day before initiation of the social interaction each fish in a pair was individually marked by a small cut in the upper or lower part of the tail fin. The location of the cut was dependent on the feeding latency score. The fish in a pair with higher feeding latency score was cut on the upper portion of the fin while in the other fish, the cut was on the lower portion of the fin. To prevent the effect of home location familiarity, the fish in a pair was introduced into a new environment (30 x 30 x 15 cm aquarium) at the same time and separated by a center divider. Body weights of dominant and subordinate individuals were recorded before and after the first encounter. Every morning, the division of the aquarium was removed for ten minutes and the social interaction of the fish was observed. After daily interaction, both fish were then fed to satiation once a day and after 14 days of pairing, the social status (behavior, eyes and body color) of each fish was measured.

Growth Rate Observation and IGF-1 mRNA Analyses

The weight of each fish was recorded weekly over the 14-day social interaction period to establish changes in growth among the dominant and subordinate fish. At the end of the study, ten pairs of fish were sampled for liver for extraction and determination of IGF-1 mRNA levels.

Total RNA from the liver was purified using Trizol® (Invitrogen™, Carlsbad, California, USA) using the protocol suggested by the manufacturer. The purity of the isolated samples was assessed by using the A260 : A280 ratio which ranged from 1.6-2.0, with most reading ranging from 1.9-2.0. The amount of RNA in ng per µl sample served as the basis for the addition of 1 µg total RNA template in the reactions. First strand cDNA was generated in 20 µl RT reactions with 1 µg total RNA template, Omniscript® reverse transcriptase, 10X RT buffer, 5 µM dNTP, 10 µM oligo-dT primer (Promega®, Madison, Wisconsin, USA), and RNase inhibitor (RNasin®, Promega®). Samples were reverse transcribed by incubation at 37 °C for 60 min. The IGF-I mRNA was quantified using the TaqMan real time quantitative reverse transcriptase – polymerase chain reaction (qRT-PCR) assay described in Vera Cruz et al. (2006) which was performed on Lightcycler® 480 II (Roche Ltd., Basel, Switzerland). Values for IGF-1 mRNA were normalized to total RNA.

Statistical Analyses

Frequency difference was analyzed using Binomial test. Linear relationships of feeding latency percentage, aggression, ECPs and durations of social interaction were assessed using Pearson correlati coefficient. Durations of appetite inhibition and interaction was analyzed using Chi square test. Statistical analyses was carried out using the Statistical Analysis Software (SAS).

Study 2 – Effect of Broodfish Social Condition on Seed Production of Nile Tilapia

This experiment was undertaken to assess the effect of broodfish behavioral stress response (BSR) on seed production of *O. niloticus* through evaluation of their feeding response score during isolation.

Determination of Social Groupings
Two potential social groupings in relation to broodfish behavioral stress response (BSR) of tilapia breeders during isolation were used in this study: the low stress response (LSR) and the high stress response (HSR) groups. The LSR breeders were those that manifested shorter period of adjustment in response to stress experienced after transfer to a new environment as indicated by a higher feeding response score (FRS). While the HSR breeders on the other hand, were those that exhibited longer period of coping with stress or those that had lower FRS. Their BSR were quantified through feeding response evaluation during the isolation period

Male and female FAC selected Nile tilapia (FaST) breeders of the same ages with body weights ranging from 75 – 100 grams were used in the study. The stocks were acquired from the FAC, CLSU, Science City of Muñoz, Nueva Ecija. The breeders were stocked for 10 days in rectangular tanks (2.30 x 1.30 x 1 m) prior to isolation. A total of 30 male and 90 female FaST breeders were used to represent all the social groups in all the treatments.

Glass aquaria measuring 30 x 60 x 30 cm were used as isolation chambers for seven days for the randomly selected male and female FaST. Each unit was provided with aeration system to sufficiently provide the dissolved oxygen requirement of the fish. Three sides of each aquarium were covered by a white plastic to avoid seeing other fish in the adjacent isolation chambers. Prior to isolation, weight of individual fish was recorded. Along with the BSR through feeding observations, the weights obtained were used as basis in the distribution of the breeders in their respective treatment assignments. This parameter was also considered in the group allocations to prevent weight from being a factor in the reproductive performances of the breeders and ensure that the results obtained were only affected primarily by their BSR.

Once fish were assigned to their respective isolation chambers/aquaria, they were hand fed once a day with a commercial diet at a rate of 1% body weight. Feeding rings were used when feeds were administered to be able to clearly observe the possible feeding response that the fish might manifest. The time the fish resumed feeding after the transfer to the new environment and the particular feeding response executed until the 7^{th} day of the isolation period were closely monitored and recorded. After the isolation period, points were summed up to get the total feeding response score (FRS). Breeders that accumulated feeding points ranging from 9 to 17 were assigned to the low stress response (LSR) group, while breeders with points ranging from 0 to 4 belonged to the high stress response (HSR) group. Since weight was also considered, the fish which demonstrated good feeding response but had higher size variations were rejected.

Feeding behavioral response manifested during the entire isolation period was quantified through corresponding points assigned for a particular feeding response as indicated previously. The scoring is as follows:

0 points - fish does not react or eat the food within a 2 minute time frame;
1 point - fish eats only pellets directly put in front of it, and does not move to eat the food other feeds;
2 points - fish moves to eat the food, but comes back to its original place in the aquarium between each feeding; and
3 points - fish eats all food present in the aquarium regardless of the position of feed pellets within the aquarium

Seed Production Evaluation
The feeding response score (FRS) obtained and breeders' weights were the bases for the social groupings. The selected breeders were distributed in the two social groupings; LSR and HSR groups, where each group was composed of 15 male and 45 female breeders. The sex ratio was one male: three females. The

breeding was done in hapas (1 x 2 x 1 m) installed in ponds with a stocking density of 4 pcs/m^2, which is typically used for breeding tilapia.

The description of the assignment of fish in their respective 5 treatment grouping is as follows:
LSRHSR♂♀ = 1 HSR male, 1 LSR male, 3 HSR females, and 3 LSR females,
LSR♂♀ = 2 LSR males and 6 LSR females,
HSR♂♀ = 2 HSR males and 6 HSR females,
LSR♂HSR♀ = 2 LSR males and 6 HSR females, and
HSR♂LSR♀ = 2 HSR males and 6 LSR females

Each treatment was replicated three times.

After the 14 days breeding period, female breeders were inspected for the presence of eggs and fry. Eggs were collected from the mouth of the female breeders by pressing the mouth of the fish and gently opening their operculum through the collector's thumb and index finger. In case of existence of swim-up fry, collection was done using scoop nets. The swim-up fry collected were temporarily placed in a plastic basin with water and immediately counted.

The parameters observed and recorded include spawning success, total seed produced, hatching rate and survival rate. The egg quality in terms of size through its diameter was also evaluated. As for the male breeders, sperm analyses through motility scoring and sperm density were also observed.

Egg Quality: From each replicate, 25 eggs were randomly selected to represent the whole population. Since the eggs were oval in shape, the diameter of each egg was carefully measured at their longest portion using a vernier caliper.

Sperm Quality: The procedure done by Danting (1992) for scoring sperm motility was followed. Sperm motility in all samples was scored on a subjective rating scale system of 0 to 10. A rating of 10 denotes that 100% of the spermatozoa under observation are motile, moving actively, while zero (0) rating indicates that no sperms are moving after activation. Prior to scoring 20 µl of sample was diluted in 100 µl water for activation. Following activation, sperm motility scoring was determined under the microscope. Only sperm swimming in a forward motion were included in estimation of motility.

Sperm density was estimated using a Neubauer slide counter (Haemacytometer, 0.1 mm., 1/400 mm^2, Weber Scientific, England). Before the milt was used for any purpose, sperm head counts were done to estimate the whole sperm sample densities. Milt was subsequently diluted and a 10 ul was dropped on the Neubauer Slide (Haemacytometer) for counting. The slide was left for approximately 10 minutes to allow the sperms to settle into one plane.

Spawning Success: The average number of eggs produced was evaluated on the 2nd production cycle, since the first cycle already contained swim-up fry. Average egg production per female was calculated as the number of eggs collected / number of female breeders that spawned. Spawning success was determined by percentage spawning rate [(number of female breeders/total number of stocked female breeders) x 100]

Hatching Rate: During the incubation period, a rate of water flow was maintained to allow a continuous movement of the eggs which also prevents their damage, clumping and settlement. Round bottom incubation jars were used, since previous studies have proven that these containers can give good results provided that water flow rates and water quality are carefully regulated (Subasinghe and Sommerville, 1992). Percent hatching rate was calculated as number of hatched eggs/total number of eggs x 100.

Fry Survival Rate and Size: When the yolk sac had been absorbed, the fry were transferred in the nursing hapas installed beside the breeding and conditioning hapas. Stocking was done in the morning. The fry were reared for three weeks or until size # 24 was obtained. Daily feeding (twice a day) of commercially available fry mash at five per cent of their body weights was done to optimize their growth. After the rearing period of three weeks, the post-fry were collected and their survivorship determined (number of post fry survived/total number of fry x 100).

The harvested post-fry for both production cycles were subjected to length and weight measurements. For the determination of their lengths, 20 samples were randomly selected for each replicate in each treatment, in each batch. Each post-fry was measured using a vernier caliper. Weight was determined through dividing the acquired collective weight of sampled post fry by the number of individual fry.

Statistical Analysis

The treatments were determined in terms of the ratio of male to female, either singly or in combination, based on their identified potential social status. The treatments were allocated in each hapa following the Randomized Complete Block Design (RCBD) with three replications per treatment. Two-way analysis of variance for RCBD was done and Least Significant Difference was used to identify the effect of behavioral stress response of the fish among the social groupings. Appropriate analysis like analysis for unequal replications and data transformation were calculated using the General Linear Model via the SAS V9.0 software.

Study 3 – Effect of Broodstock Social Condition on the Growout Performance of Nile Tilapia Fingerlings

There were two phases in this study, the first phase was the determination of the social condition of the broodfish by isolation for 5 days in aquarium and observing feeding response. The feeding response was done once a day for the entire 5 days of isolation and quantified through the corresponding points assigned for a particular feeding response, as previously described above. The breeders that obtained total points ranging from 9 to 15 were assigned to the low stress response (LSR) group while those that had 0 to 8 were considered the high stress response (HSR) group. After determining the broodfish with LSR and HSR, males and female breeders of the same social condition were conditioned separately in conditioning hapas prior to the breeding period. After the breeding period, fry were collected after 15 days and were stocked for sex-reversal treatment in hapas for 21 days. Sex-reversed fingerlings were used for the second phase of the study – the grow-out period.

The second phase of the study was composed of two treatments that were replicated three times. Treatments were as follows: I – sex-reversed fingerlings produced from LSR broodfish, and II - sex-reversed fingerlings produced from HSR broodfish. Size 22 (ave. wt. ranged from 0.192 to 0.208 g) of sex-reversed Nile tilapia fingerlings were used in the study. Fingerlings were stocked in six 200 m^2 earthen ponds at 2 fish· m^{-2}. The fingerlings were fed twice a day with commercial feeds at 20% of the body weight from 0-2 weeks, 10% of the body weight from 2-4 weeks, 7% of the body weight from 4-6 weeks, 6% from 6-8 weeks, 5% from 8-10 weeks, 4 % from 10-12 weeks.

Regular fertilization was made using inorganic fertilizers such as ammonium and 5.6 kg P·$ha^{-1}week^{-1}$ to enhance the growth of natural foods in pond water. Fertilization of ponds was dependent on the productivity of the pond water. Secchi disc visibility readings were maintained at 40 cm and below.

Eighty individuals were sampled biweekly to obtain average weight and length using cast net method as a sampling device to check the growth of stocks. At the end of the culture period, 80 fish or 10% of the total fish stock were sampled for individual weight and length.

Water quality parameters such as dissolved oxygen, water temperature, pH, total ammonia-nitrogen; alkalinity and phosphorus levels were monitored weekly starting at 9 AM in the morning.

After 90 days of culture period, all data were gathered and analyzed by T-Test in randomized complete block design with three replications.

Study 4: Effect of Stocking Density on the Growth Responsiveness, and IGF-1 Expression in Nile Tilapia

Along with rapid expansion and intensification, there is a growing concern on the welfare and health of farmed fish. Vahdatpour et al. (2009) and Mostl and Palme (2002) pointed that stocking densities in commercial aquaculture have been highlighted as a subject of increasing importance as far as fish health is concerned. Any alteration in the physical and psychological state of a living organism as it interacts and responds to environmental variations (Chandroo et al., 2004) like rearing at higher stocking densities induce stress. With aquaculture's expansion, more accurate information on stress control is highly necessary in order to assure health of fish. Scientific investigations have shown interest on early detection of stress in fishes that has led to increased study of potential biochemical, subcellular, cellular, histological and behavioral markers or biomarkers of stress.

This experiment assesses the effects of stocking density on *O. niloticus* growth and survival, hepatic IGF-I gene expression and stress responsivenss associated with stocking densities. We initially intended on measuring cortisol as a marker of stress in this study, but the plate reader intended for its measurment at CLSU was in disrepair and required substantial modification to measure plasma cortisol by an ELISA validated by our group (Cayman, Ann Arbor MI. USA). An alternative approach would be to measure cortisol by a radioimmunoassay (Dean et al. 2003). However, this technique requires isotopes not permitted for use at CLSU. Also, because of substantial time required for shipment and delicate nature of the samples they could not be reliably sent to NCSU for measurement. Nevertheless, we modified our objective to measure not only survival, growth and hepatic IGF-1 gene expression, but various other possible stress sensitive parameters including hematological, glucose, hepatosomatic and cholecystic indices. As good management provides the key to the avoidance of essentially all health problems whether stress related or not measurement of these variables will both establish reference ranges for Nile tilapia and those potentially linked to stress that could be used to assess overall health status of fish.

Rearing and Conditioning of Fingerlings in Net Enclosures

Seven (7) conditioning enclosures "hapas" with measurements of 2 x 5 x 1 m (10 m^3) were installed in a 1000 m^2 pond at the FAC-CLSU. Stocking density for each conditioning hapa was 150 pieces/m^2 or 1,500 pieces/hapa. The first two nets were stocked with mixed sex tilapia and the remaining five nets were stocked with sex-reversed fish. Fry mash was given twice daily, with a feeding rate of 20% of the fish biomass on the first week, and adjusted to 11% on the second week. On the third week, the feeding rate was lowered to 10% of the fish biomass and on the last week of rearing, the feeding rate was lowered to 6.5%. A support set of fingerlings were also reared and conditioned in 200 m^2 pond at the Genetic Improvement of Farmed Tilapias-FeedMix Fortified ($GIFT_{FF}$) facility at CLSU. The stock included 20,000 pieces of size 20 fingerlings with an average weight of 0.6 grams. Fingerlings were fed 15% biomass on the first week, 13 % on the second week, 10% on its third week and 9% on the last week. Growth rate was monitored weekly.

After acclimatization, 8,000 experimental fishes were distributed randomly in four treatment groups and each treatment was replicated four times. Fish were fed 2% of the biomass. All treatments were set in 16 experimental units following a Completely Randomized Design (CRD). The treatments used were as follows:

T1 - 250 fish/m^3, control, low density, mixed-sex
T2 - 250 fish/m^3, low density, sex-reversed males
T3 - 500 fish/m^3, medium density, sex-reversed males
T4 - 1000 fish/m^3, high density, sex-reversed males

Fish weights were obtained from fish at the beginning and end of the experiment. SGR was also determined over the course of the experiment by obtaining fish weights at 10 (March 19), 24 (April 2), and 30 days (April 8 – end of experiment)

Water Quality Monitoring: The temperature ($^{\circ}$C) and dissolved oxygen (mg/li) were monitored in the morning and in the afternoon during the 30-day nursery period. Water transparency was determined using a Secchi disk. The average Secchi disk readings were used in the calculation of Trophic State Index (TSI = 60-(14.41*[Ln (average Secchi disk readings)]).

Hepatic IGF-1 mRNA: Fingerlings from net enclosures and ponds were drawn for the initial, basal measurement of hepatic IGF-1 mRNA. These samples represented the following: three females and three males from mixed-sex fingerlings reared in hapa, three males from sex-reversed fingerlings reared in hapa, and three males sex-reversed fingerlings reared in ponds.

For the experiment, a total of 48 juvenile fish samples were collected for IGF-1 determinations from each replicate at 7, 15 and 30 days (Mar 16, Mar 24 and Apr 8) from initiation of the experiment. The weight of each fish sample was taken and recorded. An incision started off at the anal region up to the abdominal part to expose the liver of the fish. Hepatic tissue samples were collected and immediately placed in 0.5 ml microcentrifuge tubes and frozen in liquid nitrogen.

Total RNA was isolated from the hepatic tissue using Trizol (Invitrogen™, Carlsbad, California, USA). Glycogen in the samples were removed using a high salt solution. Two reaction cycles were done on RNA samples to remove possible genomic DNA contamination using DNA-free™ (Ambion, Austin, Texas, USA). The RNA concentration was quantified by spectrophotometry at 260 nm and its purity was checked by obtaining the 260/280 ratio which ranged from 1.90 to 2.05 (NanoDrop spectrophotometer, MSI U 100 Series, Wilmington, DE, USA).

Sample RNA was reverse transcribed to produce cDNA. This cDNA was subsequently measured by TaqMan® real time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) as previously described (Vera Cruz et al. 2006). A serial dilution of cDNA was run to generate a standard curve (the log of initial target copy number was plotted against the threshold cycle) of IGF-1. The amount of IGF-1 mRNA in each sample was calculated by substituting the generated threshold cycle values to the equation derived from the standard curve (Bustin 2002). IGF-1 mRNA (ng cDNA) was normalized to sample total RNA (ul total RNA).

Hepatosomatic and Cholecystic Indices: Samples of the fish's liver and gall bladder were weighed and recorded from each replicate after the 30-day nursery period. The hepatic tissue and gall bladder were collected after dissection then weighed and measured using an electronic scale (SANFORD 1261552, 0.01g; 2000 capacity). The hepatosomatic (liver weight/body weight x 100) and cholecystic (gall bladder weight/liver weight x 100) indices were measured

Hematological and Blood Glucose Profiles**:** Blood samples from each replicate were drawn by cardiac puncture using 1cc tuberculin heparinized syringe. Blood sample from each replicate was pooled in heparinized 1.5 ml tubes and placed temporarily in crushed ice. Whole blood glucose was determined using a glucose meter (Glucostar-Glucometer). Total red blood and white blood cells (WBC) were

counted using- Hemacytometer ((Neubauer,USA). The Turk solution for WBC counting was prepared using 0.05 g gentian violet, 1 ml glacial acetic acid and 100 ml distilled water. The Gower solution for RBC counting was prepared by mixing 9.38 g sodium sulfite anhydrous, 25 ml glacial acetic acid and 150 ml distilled water.

Rearing of Fingerlings in Tanks
Parallel investigations were initiated in semi-intensive tanks at the NCSU Pamilco Aquaculture Field Station (Aurora, NC). Sex-reversed Nile tilapia for this study were stocked in 1500 L tanks at a density 5, 10 and 30 kg m^{-3}. Following a 3-week growth period, the experiment was terminated by hurricane Irene in August 2011. PAFL was highly damaged and lost its dormitory and emergency backup generator from massive flooding. The fish from the experiment did not survive.

Statistical Analyses
Data on hepatic IGF-1 mRNA, growth, hepatosomal, cholecysctic, glucose and hematological indices from each replicate of each treatment are expressed as mean ± standard deviation. All data were analyzed statistically by General Linear Models procedure (SAS 1999). Tukey's Multiple Range (5% probability level) was used to test the differences between treatment means. A paired comparison using Proc Means of the Statistical Analysis System (SAS) was also done on variable weight, survival and IGF-1 to test whether there is a significant difference prior to and post stress evaluation of fish reared in different densities.

RESULTS AND DISCUSSION

Study 1 - The influence of duration of behavioral stress response on social dominance in tilapia

Phase 1 - Isolation and monitoring of the duration of appetite inhibition (DAI)
DAI after transfer to isolation units:
The mean DAI for all isolated fish was 83.55 ± 14.29 (mean ± SEM) minutes. The shortest DAI was 0.31 minute and the longest was 570.76 minutes. After the matching pairs for later pairing had been established, short DAI group had a mean DAI of 33.55 ± 10.15 minutes, which was significantly shorter ($P < 0.01$) than that of the long DAI group (133.54 ± 22.86 minutes; Figure 2).

Social interaction:
During the introduction of competing individuals in the aquarium, both fish displayed pale body coloration with dark stripes. The mean duration before observance of first attack was 10.86 ± 2.13 minutes. The fastest individual to adapt to the social condition and that attacked the opponent took less than 6.0 seconds, while the longest duration before observance of first attack was 33.66 minutes. However, at the beginning of the social encounter it was not always the fish with shorter DAI that initiated the fight. Thirteen (52%) of the 25 fish with shorter DAI (compared to their respective opponents) initiated the fight, while fish with a longer DAI initiated 11 social interactions. One pair did not show any interaction.

During the social encounter, the dorsal fins of both fish were raised and both swam towards each other indicating their preparedness to fight. Then they began aggressive interaction which involved chasing, rapid circling and biting directed against the mouth, fins and other body parts of the opponent. During this period of intensive interaction, both fish exhibited pale body stripe coloration. However, during the later part of the interaction, challenged fish mostly rebuffed attacks and eventually one of the fish chased and bit the flanks of the other fish that was fleeing. At this point, aggressive behaviour became unidirectional, and an aggressive dominant individual and a retreating subordinate fish was clearly identified. It was also observed that body- and eye-darkening of the fish increases with subordination and declines with dominance.

Overall, formation of a stable dominant-subordinate relationship was observed in 24 out of the 25 tested pairs for social dominance. Seventeen dominant fish (70.83%) of the 24 had shorter DAI during isolation compared to their opponents. This frequency difference on DAI of the dominant individuals was significant (Binomial test, $P = 0.03$; Figure 3). However, as previously mentioned, social encounter was not always initiated by the earlier eaters (i.e. shorter DAI), but eleven (64.70%) of the 17 dominant earlier eaters initiated the fight and the remaining six individuals did not start up attacking the opponent yet won the fight. On the other hand, five later eaters that became dominant began the fight, while the remaining two did not.

The recorded mean number of attacks of the 24 pairs before winning the fight was 73.33 ± 14.31. The most aggressive pair had 201 attacks in a 10-minute fight and had 277 attacks in the whole course of interaction. By contrast, the least aggressive pair made no more than one attack before the establishment of dominance. The DAI difference and level of aggression (number of attacks) between the competing pairs showed an insignificant weak positive correlation ($r = 0.28$, $P = 0.193$).

Dominance/Subordination and Growth

Reduced growth rate is a well-described physiological end result of social stress. The mean weight of subordinate fish before the interaction was 26.17 ± 1.40 g and this was reduced to 23.29 ± 1.36 g one day after the fight. While in the dominant fish, average weight was decreased from 26.81 ± 1.45 g to 24.70 ± 1.36 g (Figure 4). The mean decrease in weight was significantly higher for subordinate fish (2.88 ± 0.21 g) compared with dominant fish (2.11 ± 0.19 g) ($P < 0.01$).

Mortality of Subordinates

Death can be the most overwhelming effect of stress. After the interaction of each pair, winner and loser individuals were easily identified by their displayed behaviors. One day after the fight, one subordinate fish immediately died followed by four on the second day, nine on the third day, which was the day with the highest number of mortality. Another three died on the fourth day, five on the fifth day and one on the sixth day. The last surviving subordinate individual died on the seventh day after the interaction. It took one week from the day after the social interaction for all subordinate fish to die.

Phase 2 - Feeding latency as an indicator of stress coping style

Feeding Response Score and Social Interaction

We found that tilapia with higher FRS during the isolation had a greater possibility to win the fight for social dominance. Formation of stable dominant-subordinate relationship was observed in 59 of the 60 pairs tested. Thirty six fish of the 59 after the first interaction and 34 of the 56 after 14 days that became dominant had higher FRS compared to that of their conspecifics (Binomial test, $P < 0.05$). This indicates that social dominance can be predicted using the FRS of the fish during isolation so long as FRS values are not very close among the pairings.

Dominance/Subordination in Relation to Growth Rate and Hepatic IGF-1 mRNA Expression

Table 1 shows a summary of time before first acceptance of food and feeding latency or response score during the isolation, and weight of competing individuals before the interaction and 1, 7 and 14 days after the first interaction. Before the social interaction there was no significant difference on the body weight between the competing individuals (see Table 1). During and after the interaction, most of the dominant individuals have higher body weight than that of the opponent.

On growth of the fish, dominant fish had a mean SGR of 1.61% which was significantly higher ($P = 0.013$) than that of the subordinate fish (0.93%). The correlation of IGF-I mRNA and the specific growth rate (SGR) of the fish from day 7 to 14 of the social interaction period is shown in Figure 5. Generally, fish with faster growth rate had a higher IGF-I mRNA gene expression. The mean relative abundance of

hepatic IGF-I mRNA of dominant fish was elevated 73% when compared with the subordinate fish (P = 0.005) after 14 days. There was a significant overall positive correlation (r = 0.65) between SGR and relative abundance of IGF-I mRNA (P = 0.002).

Behavioral stress response can be used to predict outcome of contest for social dominance. Results of the present study indicate that tilapia with shorter DAI after its transfer to isolation in a new environment has a greater chance to become dominant when paired with another individual. The results that fish with low behavioral stress response became dominant in majority of the social pairing are similar to those established for the anole and rainbow trout (Korzan et al. 2006; Øverli et al. 2004; Pottinger and Carrick 2001). The time variation of resumption of food intake (ranging from seconds to hours) of fish after being transferred to new environment most likely reflects some aspects of the physiological stress responses to confinement, which could also affect the outcome of the social interaction (Øverli et al., 2004). According to Bernier (2006), stress induced inhibition of food intake in fish may, in part, be mediated by corticotrophin-releasing factor system which plays a key role in controlling the neuroendocrine, autonomic, immune, and behavioural responses to stressors. On the other hand, the fish's resumption of feeding after having coped with a stressful condition may reflect a down regulation of the physiological stress response (Øverli et al. 1998).

The results that not all fish with shorter DAI won the fight calls for a need to refine the method of assessing the behavioral stress response in this species of fish. In a review, Øverli et al. (2007) described how feeding behavior can be used as an indicator of stress coping style. Feeding behaviour can be assessed using point system based on the feeding behavior of the fish when fed daily for one week during isolation. In phase II of this study, we evaluated the FRS over a 10-day isolation period and found that social dominance can be predicted from animals that exhibit a higher FRS so long as FRS values among pairings clearly differ and are not too close to each other.

Social encounter is potentially costly and risky to the fighting opponents. The cost of fighting includes energy, time and physical injuries. The individuals engaged in social fight are integrating the costs and benefits associated with the contest and adjust their behavior accordingly (Hsu et al. 2006). At a certain point when an individual in a pair reaches its own dangerous threshold, an established dominant-subordinate relationship will be observed after one of the fish retreats or surrenders. In the current study, the observed changes in behavior and body and eye color of the competing fish served as social signals to the opponents to limit aggressive interaction. When social hierarchy had been established, subordinates showed increased body stripes and eye-darkening patterns relative to dominant fish which showed decreases in these variables. These observations conform with previous work (Volpato et al. 2003; Bero 2008; Vera Cruz and Brown 2007).
Social aggression is stressful for both dominant and subordinate fish (Summers and Winberg 2006). In social interaction, defeat in many animal species is a powerful stressor that can lead to drastic alterations in physiology and behavior. Behavioral effects of defeat include appetite inhibition (Gómez-Laplaza and Morgan 2003; Øverli et al. 1998; Winberg et al. 1993), reduced aggression (Höglund et al. 2001; Blanchard et al. 1995), and increased submissive and defensive behaviors towards conspecifics (Blanchard et al. 1993; Siegfried et al. 1984). The observed weight reduction in the current study after the interaction in both the dominant and subordinate fish supports our previous findings (Vera Cruz and Brown 2007). The reduced weight of subordinate fish a day after the social interaction may be more a result of appetite inhibition rather than a reflection of mobilization of stored energy for physical activity associated with social stress encountered. The subordinate fish were observed not consuming food after the social interaction and dominant fish even guarded or monopolized the food against the opponent. On the other hand, the increased physical activity of dominant fish during and after the aggressive encounters, a behaviour indicating that they have won the contest, may have contributed to the lower mean weight of the fish after the interaction. However, during the establishment of social hierarchy, the two social groups experienced similar level of physical activity. Thus, body weight differences between

the two social groups during the establishment of social hierarchy were mainly attributed to physiological and behavioral differences such as appetite rather than to differences in physical activities (Fox et al. 1997; Øverli et al. 1999). Inhibited food intake in subordinate fish may be due to social stress-induced increase in the serotonergic activity in the brain (Winberg et al. 1992) and/or neuropeptide Y mRNA expression in the preoptic area (Doyon et al. 2003).

The mortality of subordinates in Phase I of the studies is most likely a result of exhaustion caused by social stress. This was also observed by Petrauskiené (1996) in rainbow trout reared at low densities (2 or 3 individuals) where most of the subordinate fish may have reached the exhausted state during the third day. Subordinate fish confined with a dominant fish experience social stress and showed increased standard metabolic rate or a metabolic disadvantage (Sloman et al. 2000) that may lead to impaired growth. Our previous work shows that lower social status depresses hepatic insulin-like growth factor-I (IGF-I) levels while dominant status stimulates IGF-I production (Vera Cruz and Brown 2007). In Phase II of the present study we found that dominant fish predicted by an elevated FRS, exhibited increased growth and that this is associated with enhanced hepatic IGF-1 mRNA levels. With social stress seen with subordinates, hepatic IGF-I declines leading to reduced growth rate.

Overall, the results of these studies suggest that DAI or FRS are good predictors of social status in Nile tilapia with shorter DAI and higher FRS values during isolation leading to a significantly greater proportion of individuals that show dominance during social interaction. The dominant individuals have improved survival, enhanced hepatic IGF-1 gene expression and increased growth rate. The opposite occurs in subordinate tilapia whose social status and reduced capacity to cope with stress can be predicted from lower feeding response scores and a longer duration of appetite inhibition during a previous period of isolation.

Study 2 – Effect of Broodfish Social Condition on Seed Production of Nile Tilapia

Egg Quality: The measured average size of the eggs collected from each treatment is presented in Table 2. Eggs from LSR♂♀ (2.86 mm) were significantly bigger than those of the other treatments. Comparison of mean egg sizes in LSRHSR♂♀ and HSR♂LSR♀ revealed no significant difference but their egg sizes were significantly higher than those in HSR♂♀ and LSR♂HSR♀, which were comparable with each other.

The average size of eggs in this study ranged from 2.16 to 2.86 which is in agreement with the findings of Payne and Collimson (1983) who showed that eggs of *O. niloticus* usually range in size from 1.94 to 2.95 mm. Although mean egg sizes in all the treatments fell under the said range, the social grouping where both the breeders manifested LSR had a significantly higher egg size than the others. Rainbow trout (*O. mykiss*) subjected to a milder stress regime during early vitellogenesis produced smaller eggs that varied in size, while there was no effect on mean egg size in fish stressed during late vitellogenesis (Contreras-Sanchez et al. 1998). It is possible therefore that stress encountered by the HSR female breeders during vitellogenesis led to the production of smaller eggs in the LSR♂HSR♀ and HSR♂♀ treatments. Also during the whole isolation period, the majority of HSR breeders did not show any aggression towards the introduced feed and did not even eat the feeds within the two-minute time frame given. These behaviors by the HSR breeders might have also been manifested during the breeding period whereby a deficiency in nutrients may have contributed to the smaller egg sizes for these social groups.

Sperm Quality: The accumulated FRS of the male breeders with their corresponding motility scores are presented in Table 3. Sixty-five males were subjected to isolation for seven days, however, only 20 of them produced sperm during the collection. Twelve males were chosen to represent the respective FRS. Eighteen was the highest FRS a male breeder was able to accumulate during the whole isolation period, while, zero was the lowest gathered FRS.

The highest motility score obtained was 10 and the lowest was 3. The samples with accumulated FRS from 6 to 10 all attained a sperm motility score of 10. However, although they all had100% motile sperms, a difference in terms of active movement was also observed under the microscope. The male breeder with a 10 FRS had 100% motility, but only 75% of them were actively swimming and the remaining 25% showed a more moderate pace of motility. While the sperm collected in the male breeders with an FRS of 6-8 consisted of 100% motile sperms, only 50% of them were active and the other half showed slow motility. The breeders with an 11 to 18 FRS had 100% motility that consisted of very active and fast swimming spermatazoa. Low sperm motility could actually reduce fertility even despite an increase in sperm quantity (Kurokura and Oo 2008). Breeders with FRS of 0-5 showed relatively low motility scores, whereby 50% of their moving sperms were actively swimming and the other half showed moderate to slow movement. The breeder that produced the lowest motility score of 3 (FRS of 0), showed only 30% motility that was of moderate pace.

Table 4 presents the sperm density for the accumulated FRS by each male breeder. The accumulated feeding score from zero to six were considered to belong to the HSR group while those that accumulated 10 points and above were under the LSR group. Breeders that gathered 15 and 18 FRS obtained the highest sperm densities of 1.063×10^{10} and 9.400×10^{9}, respectively. On the other hand, zero and one FRS had the lowest densities of 2.025×10^{9} and 3.100×10^{9}, respectively. Male breeders that were able to accumulate 18 and 15 points had the significantly higher sperm densities than those of the other breeders. While, the breeder with FRS of zero had significantly lower sperm density compared to those of the other breeders with higher FRS. The trend shows that the FRS was directly proportional to the sperm density; the higher the accumulated FRS of a male breeder, the more sperm it produces. In the present study, LSR breeders show dominance over the fish that belong to the HSR group and show higher sperm density and motility. Hence, dominance may increase the sexual maturation rate in Nile tilapia males (Goncalves-de-Freitas 1999). In the African cichlid, *Astatotilapia burtoni*, social status determines reproductive capacity of males via increased activity of the of the brain-pituitary-gonad (BPG) axis, which leads to increased production of sex steroid hormones (Parikh et al. 2006; Burmeister et al. 2007). In the hypothalamus, gonadotropin releasing hormone (GnRH) stimulates the secretion of gonadotropins which stimulate male reproductive functions and secretion of testosterone hormone required for spermatogenesis and sperm transport (Bearden and Fauquay 1980). In bulls, previous studies revealed that GnRH treatment increases sperm concentration and levels of live spermatozoa (El-Azab et al. 1996; Gabor et al. 1998). Considering dominance increases the activity of the BPG axis in cichlids, which leads to enhanced production of gonadotropins, it is quite plausible that breeders with increased FRS and that manifest low stress response behaviours (show dominance) are the ones that show increased sperm density and motility.

Spawning Success: Table 5 presents the average spawning success per treatment during the 2nd production cycle while Table 6 presents the average number of eggs a female was able to produce in one spawning. Spawning success in LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ were comparable and significantly higher than that in the HSR♂♀ group. The average number of eggs produced per female in the LSR♂♀ group was significantly higher than those in LSRHSR♂♀, LSR♂HSR♀ and HSR♂♀ but comparable with that in HSR♂LSR♀. The HSR♂LSR♀ group revealed no significant difference with that of LSRHSR♂♀, but was significantly higher compared to the LSR♂HSR♀ and HSR♂♀ groups. The LSRHSR♂♀ and LSR♂HSR♀ on the other hand were comparable. Average number of eggs produced in HSR♂♀ was significantly lower than those of the other treatments.

All treatments with both or either male or female LSR breeders (*i.e.* LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀) had comparable spawning rates that were significantly higher ($P < 0.05$) than those in the HSR♂♀, which was composed of HSR male and female breeders. However, the advantage of treatments that consisted of LSR female breeders (LSR♂♀, 951.67 ± 151.28 and HSR♂LSR♀, 797.00 ±

129.26) was that they produced more eggs per female per spawning than those in the groups with HSR female breeders ($P < 0.05$). Interestingly they also had increased egg size (Table 2).

Eggs and Fry Production: The total number of eggs and swim-up fry for the 1st and 2nd production cycles is presented in Table 7. The swim-up fry collected in the 1st production was added to the number of the eggs collected; it was assumed to have a 100% hatching rate.

Breeders from LSRHSR♂♀ produced the highest mean number of eggs for both cycles having 3260.33 and 1883.67 eggs, respectively. It was followed by those in LSR♂♀ with 2484.33 eggs in the 1st cycle and 1582.33 eggs in the 2nd cycle. The breeders in LSR♂HSR♀ obtained a higher mean egg collection of 1557.33 eggs in the 2nd cycle than in its 1st production cycle which was just 867.33 eggs. This same trend was also shown in HSR♂LSR♀. On the other hand, breeders in HSR♂♀ generated 2142.00 eggs in the 1st production, however, this figure was only a representation of just one replicate. No eggs were collected in the other two replicates. And for the 2nd production, no eggs had been collected in all the replicates, thus, obtaining a zero (0) value. Only 1 replicate in HSR♂♀ was able to produce eggs during the 1st cycle and none in the 2nd. Three breeders had died in the HSR♂♀ group a day before the scheduled collection of the 2nd cycle; two from replicate one (one male and one female) and one female from replicate three.

On the number of eggs collected no significant differences were observed in LSRHSR♂♀, LSR♂♀, and HSR♂♀. The mean number of eggs collected in LSR♂HSR♀ and HSR♂LSR♀ were also comparable to each other, but were significantly lower than those of the first three treatments. For the 2nd production cycle, since no eggs were collected in HSR♂♀, analysis was done based on the log (x+1) transformation. Comparison among treatment means showed that those in LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀, were all comparable among each other but significantly higher than that in HSR♂♀.

Total seed produced in LSRHSR♂♀ (5144.00) was comparable to that in LSR♂♀ (4016.66), but was significantly higher than those of the other treatments (Table 8). Total seed production in the LSR♂♀ group was significantly higher than that of HSR♂♀, but comparable to those of the LSR♂HSR♀ and HSR♂LSR♀. HSR♂♀, LSR♂HSR♀, and HSR♂LSR♀ had comparable total seed production over the two cycles.

Hatching Rate: The eggs in LSR♂♀ reflected the highest hatching rates of 92.44% and 84.13% for 1st and 2nd production, respectively (Table 9). It was followed by LSR♂HSR♀ with 87.68% for the 1st production and 83.60% for the 2nd production. The LSRHSR♂♀ although having the highest number of eggs collected dropped to 3rd, as it only showed 78.26% and 83.56% hatching rates. This finding suggests that most of the eggs collected in LSRHSR♂♀ died before they hatched. Significance on the comparison among treatment means was seen only in HSR♂♀ in 1st and 2nd productions. While LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ were comparable with no significant differences.

Survival Rate: The highest mean survival rate was attained by post-fry in LSR♂♀ with 81.83% in the 1st cycle and 78.14% in the 2nd cycle (Table 10). While, the lowest rate was obtained by post-fry in HSR♂♀ with 47.53% in the 1st cycle. The post-fry in LSRHSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ on the other hand, obtained average mean survival rates of 68.64%, 66.46%, and 58.44%, respectively, in both cycles. The HSR♂♀ was significantly lower than the other treatments. While LSR♂♀, LSR♂HSR, LSRHSR♂♀ and HSR♂LSR♀ had comparable survival rates and revealed no significant differences. In the 2nd cycle, the survival rate of post fry in LSR♂♀, LSRHSR♂♀ and LSR♂HSR♀ were comparable to each other and significantly higher than that of HSR♂♀. However, that of HSR♂LSR♀ was comparable to that of LSR♂HSR♀, but was significantly higher than that of HSR♂♀. Campbell et al. (1994) found that a relatively prolonged and severe stress in rainbow trout negatively affects progeny survival. The reduced progeny viability might be attributed to limited energetic reserves allocated to the eggs as well as

mechanical damage caused by the stressor (Schreck 2000). In this study, treatments with LSR males as in LSRHSR♂♀, LSR♂♀ and LSR♂HSR♀ showed better survival percentages than in the treatments with HSR males (HSR♂♀ and HSR♂LSR♀). Highest survival rate was attained in treatment with both LSR male and female breeders. The quality of breeders may have contributed to the quality of gametes produced and resulted in better quality fry which led to higher rates of survival. The lowest survival rate of post-fry was attained in the treatment with both HSR male and female breeders.

Fry Length and Weight: After three weeks of rearing period, the swim-up fry were collected from the nursing hapas. The final length and weight of the post-fry in each treatment after three weeks are presented in Tables 11 and 12. Post-fry in LSR♂♀ group had the highest measured mean length of 20.91 and 20.34 mm for both production cycles, respectively. This was followed by those in HSR♂LSR♀ which had 19.38 mm in the 1st cycle and 19.24 mm in the 2nd. Post-fry in LSRHSR♂♀ and LSR♂HSR♀ on the other hand, obtained 18.98 mm and 18.32 mm in the 1st production, while 18.37 mm and 18.29 mm in the 2nd production, respectively. The lowest value was obtained in HSR♂♀ which was 13.88 mm in the 1st production. The LSR♂♀ had significantly higher mean length than other treatments. The LSRHSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ had comparable post fry lengths but were significantly higher than that in HSR♂♀. As for the 2nd cycle, mean length in the LSR♂♀ group was significantly higher than those of the other social groups. The length of HSR♂LSR♀ fry was also significantly higher than those of the remaining three treatments. Whereas, the mean length in LSRHSR♂♀ and LSR♂HSR♀ were comparable to each other but were significantly higher than that in HSR♂♀. Similar differences in mean weight of fry was also observed among the groups.

Overall, the highest length and weight was seen in the post-fry produced by both LSR breeders (LSR♂♀). The quality of the progeny produced is likely a reflection of broodstock quality. The LSR breeders during the isolation period showed high feeding response scores, and take a shorter period adjusting to stress as exhibited by immediate aggressiveness towards the feed provided. Along with this result, it was observed that good numbers can also be acquired as long as both the breeders were not under the HSR group. In this study, HSR♂LSR♀ with low stress response females and high stress response males produced bigger post-fry after three weeks than in LSRHSR♂♀ with equal combination of low and high stress response male and female breeders and LSR♂HSR♀ with low stress response males and high stress response females. This trend was probably influenced by the quality of female breeders having the low stress response as determined through feeding response observations.

Study 3 – Effect of Broodstock Social Condition on the Growout Performance of Nile Tilapia Fingerlings

The effect of the social condition on the grow-out performance of Nile tilapia fingerlings were evaluated by monitoring growth and performance of the fish stocks in earthen ponds produced from broodfish that show a low stress response (high feeding response scores during isolation) and high stress response (low feeding response scores during isolation). Figure 6 shows the growth pattern of both treatments in terms of the average body weight of the fish stock during the culture period.

The figure shows a comparable growth between treatments during the first month of the culture period but treatment means started to differ on the second month until the end of the experiment. Fish stock from Treatment I which was from the broodstock with low stress response obtained a higher final mean weight of 111.27 g as compared to those produced from the high response group with a final mean weight of 90.31 g. However, analysis of variance showed no significant d ifference among treatments at 5% level of significance (Table 13).

During the growout period, fish produced from the broodstock with low stress response generally obtained better results as compared to the fish produced from high stress response. In terms of feed

conversion ratio (FCR), Treatment I had a lower FCR of 1.4 as compared to Treatment II with 1.7 at the end of the culture period. The low stress response group also had the higher extrapolated gross yield (1596.7 kg/ha versus 1221.7 kg/ha. On the other hand, survival of both treatments was almost identical. Despite the trends toward improved performance in fish derived from low stress response broodfish, the difference was not statistically significant, at least, within the 3-month culture period.

Monitoring of water quality parameters was carried out throughout the duration of the study and results are presented in Table 14. The data gathered for dissolved oxygen in both treatments were in the ideal range for tilapia culture, although minimum reading of 1.8 was recorded, still, tilapia are known to tolerate low level of dissolved oxygen level but can affect growth if exposure occurs over a long period (Boyd 1990). The minimum and maximum readings for all other water parameters, including temperature, pH, ammonia-nitrogen, alkalinity, and phosphorus were desirable and similar between treatment groups. This is further underscored by the limited mortality seen in the experiment (> 70% survival).

Study 4: Effect of Stocking Density on the Growth Responsiveness, and IGF-1 Expression on Nile Tilapia

Water Quality: The temperature readings obtained in this experiment were within the optimal range of 20 to 35 °C. The morning dissolved oxygen concentrations ranged from 2.36 to 4.14 mg/l while the afternoon readings of dissolved oxygen ranged from 2.04 to 7.89 mg/l. The Carlson's technique of log transformation and calculation of the average Secchi disk readings gave a TSI of 63.95, which indicates the pond used in this study had sufficient nutrients to support fairly high natural productivity.

Growth Parameters and Survival: Survival rates ranging from 61 to 70% did not differ among sex-reversed tilapia reared at the different densities. Most of the observed mortalities occurred over the first 10 days following stocking.

At the initiation of the study average body weight and variation in weight was highest for the low density mixed sex group (control) relative to all other groups that consisted of sex reversed fish at similar or higher stocking densities. At harvest, the average final weight declined with increased stocking density. The low density (250 fish/m^3), mixed-sex (T1, 9.0 g) and sex-reversed (T2, 6.01g) had similar final body weights with significantly lower values for sex-reversed fish stocked at 500 (T3, 4.93 g) and 1000 (T4, 4.66 g) fish/m^3 ($P < 0.05$; Table 15). The SGR of fish at different sampling periods in response to stocking density-related stress are shown in Table 16. The low density, sex-reversed fish (T2) had highest SGR over the course of the experiment. Graded declines in SGR was seen as stocking density increased. This difference was statistically significant during the second sampling interval (day 10-24). The overall SGR was 8 - 10 times greater in low density sex-reversed fish relative to the medium and high density group. Interestingly, the mixed-sex low density control (T1) had a lower SGR than the low density sex-reversed male fish, suggesting energy may have been diverted to both gonadal development, particularly for females, as well as to growth in the mixed-sex population. The SGR observed in this study with lower density conforms with that shown previously (SGR of 1.7) in monosex fish reared in hapas (Little et al. 2003). The findings of Chakraborty and Banerjee (2010) on SGR of fish in ponds was higher at 5.01%, in cages at 4.68%, and in cisterns at 4.8% with additional dietary protein sources.

Data generated in this study demonstrated that fish stocked at the lowest density had better growth performance in terms of average weight and SGR over a 30-day nursery period. This was followed by sex-reversed fish stocked at the medium density with lowest performance occurring in fish stocked at the highest density. The results suggest that at higher densities the carrying capacity of fish may be limited due to space, which in turn, elicits a stress response accompanied by lower growth rate.

Hepatic IGF-1 Gene Expression: Baseline hepatic IGF-1 concentrations were recorded from the experimental fish reared in normal conditions. The IGF-1 mRNA concentrations of Nile tilapia were as follows: female in hapa (30.52 ng/µl), male in hapa (M-H, 31.5 ng/µl), sex-reversed in hapa (SR-H, 30.67 ng/µl) and sex-reversed in pond (SR-P, 29.31ng/µl). These IGF-1 mRNA concentrations served as pre-stress reference values for the succeeding IGF-1 evaluations in fish from the density study.

The levels of hepatic IGF-1 mRNA in Nile tilapia following 7 days (March 16 sampling) of rearing at different stocking densities are shown in Table 17. Hepatic IGF-1 mRNA levels was highest in low density, sex-reversed fish (T2, 31.59 ± 6.94 ng/µl) followed by the control, low density mixed-sex (T1, 27.03 ± 5.24 ng/µl). These levels were similar to those pre-stress baseline values measured in fish in hapas and ponds ($P > 0.05$). Significantly lower IGF-1 mRNA concentrations were found in medium and high stocking densities with 14.44 ng/ µl ± 10.90 and 17.79 ng/µl ± 7.64, respectively ($P < 0.05$). Due to technical difficulties and/or RNA degradation we were unable to detect any IGF-1 mRNA taken from liver on the 15 day midpoint and 30-day endpoint sampling. A post-hoc analysis in low density, sex-reversed fish (T2) on pre-stress and post-stress comparison showed that there was no significant increase in the level of IGF-1 mRNA ($P>0.05$).

Insulin-like growth factor -1 (IGF-1) is the primary mediator of the growth promoting actions of growth hormone (GH) and its levels correlate well with growth in tilapia and other fishes and vertebrates (Picha et al. 2008a). It is an indirect measure of the average amount of growth hormone (GH) being produced by the body, thus, IGF-1 mirrors GH excesses and deficiencies, making it a useful indicator of average GH levels (American Association for Clinical Chemistry 2011). The lower hepatic IGF-1 gene expression in fish reared at the medium and high densities along with their reduced growth rates clearly indicates that density-dependent stress is likely suppressing growth through inhibition of IGF-1 and perhaps GH cell activity. Brockmark et al. (2007) showed that salmonid smolts maintained at low density had higher levels of IGF-1 than those reared at high density, which is consistent with the results shown here. They further pointed out that fish kept at low density were more silvery in color and had a lower mortality rate than fish reared at high density.

Hepatosomatic and Cholecystic Indices: The liver of fish from the control, low density mixed-sex (T1) had the highest weight of 0.20 g, followed by low density, sex-reversed (T2, 0.11 g; $P < 0.05$; Table 18). The liver weight of fish from the medium density, sex-reversed (T3) and high density, sex-reversed (T4) was significantly lower at 0.08 and 0.07 g, respectively compared with low density fish. The length of liver in the control, low density, mixed-sex (T1) was longer at 3.24 mm compared to sex-reversed fish reared at the different densities ($P < 0.05$), a likely reflection of the larger body size of this fish both at the beginning and end of the experiment. There were no differences in liver length among sex-reversed fish held at the different densities. The HSI or ratio of liver weight to body weight, is a general indicator of energy reserves, namely glycogen and fat. Fish tend to have a lower HSI in a poor environment where limited nutrients might be available or where excessive energy is utilized. In gilthead seabream high stocking density reduces hepatosomatic index (from 2.26 down to 2.04)(Montero et al. 1999). The HSI values ranged from 0.961 to 1.10 in this study and there were no significant differences in the across density classes ($P > 0.05$), suggesting energy (or food) was not a limiting factor or that metabolic rate was not sufficiently elevated in fish held at higher densities, despite their lower growth rate.

The size and fullness of the gall bladder is indicative of feeding status in fish. A large, distended bladder indicates a fish that has not eaten for some time while an empty, flabby bladder suggests a recent meal (Bowen 2001). An elevated cholecystic index (gall bladder:liver weight ratio) may be suggestive of bile retention, a decrease flow of bile and therefore susceptibility to cholelithiasis. Although gall bladder weight was slightly elevated in the mixed-sex, low density fish, no differences were observed in weight, length or cholecystic index among the sex-reversed fish held at different densities (Table 18). The

highest cholecystic index of 41.3% was recorded in high density, sex-reversed (T4) fish, but this was not significantly different from the other groups.

Blood Glucose: Blood glucose levels generally rise with stress in most vertebrates to provide the necessary energy for metabolism, muscle activity and other functions needed for short term and long term adaptation to stress or the fight-or-flight response. In tilapia social stress leads to elevations in glucose relative to pre-stress levels (Porchas et al. 2009). Likewise, in sturgeon glucose levels rise following a stressor similar to that observed for bass with temperature and confinement stress (Solati and Falahatkar 2007; Porchas et al. 2009). However some studies in fish reported a weak elevation of glucose (Davis and McEntire 2006), while others found no change (Jentoft et al. 2005) and even a decrease in glucose levels (Wood et al. 2005). In the present study, sex-reversed tilapia stocked at the medium density had signifcantly higher levels of blood glucose (94.50 mg/dl) relative to the low density (62.75 mg/dl) and high density (49.75 mg/dl), sex-reversed groups ($P < 0.05$; Table 19). The use glucose as a putative marker of density-dependent stress in tilapia may be inconclusive because tilapias tend to tolerate low glucose levels and maintain their blood glucose within a relatively narrow range. This may be attributed to tilapia being omnivorous tropical species as compared to carnivorous cold water species. Alternatively, we may have missed potential changes in glucose that may have occurred in response to the initial effects of stocking density.

Hematological Profiles: Total red blood cell (RBC) and white blood cell (WBC) profiles in response to different rearing densities are shown in Table 20. Fish in the control, low density, mixed-sex group had the highest RBC count of 3.7×10^9 cells/ml of blood ($P < 0.05$). The RBC count in sex reversed fish declined in parallel with elevations in density, such that the low density group had an RBC count of 2.85×10^9 cells, the medium density 2.45×10^9 cells, and the high density 2.0×10^9 cells per ml of blood ($P < 0.01$). A virtually identical pattern was observed with WBC counts. Clearly, RBC and WBC counts decline with increasing density. A decrease of RBC count most likely suggests that higher density fish lack sufficient oxygen or our anemic despite adequate amounts in rearing water. This may contribute to lower growth rate associated with the stressor of higher densities. The variation in total WBC clearly indicates it is a good measure of density dependent stress responses. The lower WBC count with higher density may reflect release of epinephrine during stress which causes contraction of spleen and could hasten WBC destruction. Destruction of WBC may weaken of the immune system (Witeska 2005). Thus the high density, sex-reversed fish group which apparently were subjected to the most stressful condition in the course of the experiment, and had the lowest WBC count, may have a weakened immune system, which could enhance vulnerability to infection. It appears since the survival rates were similar among groups that this downstream effect was not fully apparent within the 30-day culture period. Had the culture period been extended perhaps mortality rates would have risen in fish at the higher densities

CONCLUSION

Through a series of studies we assessed if physiological and/or behavioral responses to stress can be used in the selection of broodstock with reproductive advantage in Nile tilapia, examined the effect of broodstock social condition on seed production and fingerling growout performance of tilapia, and evaluated the effects of stocking density on fingerling growth in nursery hapas, gene expression of IGF-1, and stress responsiveness of tilapia.

In the first study, we investigated whether the outcomes the outcomes of competition for social dominance among Nile tilapia individuals can be predicted by evaluating the duration of appetite inhibition or the feeding response score (FRS) after transfer to isolation. In addition, it also investigates if eye color pattern (ECP) is related to the duration of behavioral stress response such as appetite inhibition. Clear establishment of dominance hierarchy was observed in 24 of the 25 pairs. From the 24 dominants, 17 (70.83%) of them have shorter DAI during isolation compared to that of their conspecifics. This

indicates that tilapia with shorter DAI during the isolation had a greater possibility to win the fight for social dominance and therefore, dominance can be predicted using the DAI of the fish during isolation. Reduced growth rate of both dominant and subordinate fish, a well-described physiological end result of social stress, were observed one day after the social interaction. The greater weight losses in subordinate fish compared to dominant fish during and after the establishment of social hierarchy were mainly attributed to behavioral differences such as appetite rather than to differences in physical activities. Similarly, we found that animals with a higher FRS could be used as a predictor of social dominance. Again, the dominant fish had higher specific growth rates, which were accompanied by elevated expression of IGF-1, a central hormone mediating growth in tilapia and other vertebrates. Based on this research, feeding responses of broodstock in isolation are good predictors of social status, such that dominant individuals can be chosen in establishing breeding pairs.

In study 2, we assessed the effect of broodfish behavioral stress response on seed production of tilapia through evaluation of their feeding response score during isolation.

Results of the study demonstrate that behavioral stress response of Nile tilapia through evaluation of their feeding response can influence the number and quality of the seeds they produce. The most number of eggs, largest egg size, highest sperm motility and sperm density, and most number of fingerlings produced were collected in treatments that had both low stress response male and female breeders in the group. Higher rates of hatching and survival were also reflected in treatments that had either a low stress response male or female in the group. These results indicate that stress responsiveness of broodstock is a good predictor of fecundity and can be used to select fish with higher seed production. Broodstock individuals with low stress responsiveness, selected based on their increased feeding responses during an initial isolation period, can improve overall seed production in hatchery operations.

A third study the effect of social condition of broodfish on grow-out performance of Nile tilapia fingerlings was evaluated. We found that a 3-month growout of sex-reversed fingerlings derived from low stress response breeders, had a higher average body weight, a better feed conversion ratio, and an overall higher yield per hectare than fingerlings derived from high stress response breeders. Although the differences between the two groups were not statistically significant after 3 months of growout the data suggests that fingerlings derived from low stress response broodstock pose an advantage over those derived from high stress response broodstock in overall production performance. This would likely be further amplified were fish growout extended to 4 or 5 months.
An additional study investigated the effect of fingerling stocking density on the growth, gene expression of hepatic insulin-like growth factor-I (IGF-I), and stress responsiveness of tilapia reared in nursery hapas. The overall effect of density as a stressor showed that low density, sex-reversed group responded well in terms of growth, specific growth rate, survival, hematological profile (elevated red blood and white blood cell counts) and IGF-1 mRNA gene expressions compared to fish reared and confined at higher densities. These data suggest that densities of 250 fish/m^3 are best for growth of fingerlings in nursery hapas, and demonstrate that density-dependent stressors impair growth through inhibition of IGF-1 production. It is clear that IGF-1 mRNA is a strong growth rate indicator in the field and may also serve as an indirect measure of stress in tilapia. This along with certain hematological parameters may allow for assessment of environmental variables that limit stress and best promote growth in tilapia aquaculture.

LITERATURE CITED

Barcellos, L.J.G., Nicolaiewsky, S., de Souza, S.M.G. and Lulhier, F. 1999. Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. Aquac. Res. 30: 437-444.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroid. Integ. Comp. Biol. 42: 517-525.
Bearden, H.J. and Fauquay, J.W. 1980. Applied Animal Reproduction. New Jersey, USA: Prentice Hall.
Bernier, N.J. 2006. The corticotropin-releasing factor system as a mediator of the appetite-suppressing effects of stress in fish. General and Comparative Endocrinology, 146: 45-55
Bero, R.M.L. 2008. Eye color as a predictor of social dominance in Nile tilapia (*Oreochromis niloticus* L.). Undergraduate Thesis. Central Luzon State University. 53 pp.
BFAR. 2006. The Bureau of Fisheries and Aquatic Resources: Overview. Paper presented at the WorldFish Center and Philippine Partners meeting. Quezon City, Philippines.28 February 2006.
Binuramesh, C., Prabakaran, M., Steinhagen, D. and Michael, R.D. 2005. Effect of chronic confinement stress on the immune responses in different sex ratio groups of *Oreochromis mossambicus* (Peters). Gen. Comp. Endocrinol.. 250, 47-59.
Björnsson, B.T. 1994. Effects of stocking density on growth rate of halibut (*Hippoglossus hippoglossus* L.) reared in large circular tanks for three years. Aquaculture 123: 259-270.
Blanchard, D.C., Sakai, R.R., McEwen, B., Weiss, S.M. and Blanchard, R.J. 1993. Subordination stress: behavioural, brain and neuroendocrine correlates. Behavioral Brain Research, 58: 113-121.
Blanchard, D.C., Spencer, R.L., Weiss, S.M., Blanchard, R.J., McEwen B. and Sakai, R.R. 1995. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology, 20: 117- 134
Bolasina, S., Tagawa, M., Yamashita, Y. and Tanaka, M. 2006. Effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae and juveniles of Japanese flounder, *Paralichthys olivaeus*. Aquaculture 259: 432-443.
Bonga S.E.W. 1997. The stress response in fish. Physiol. Rev. 77 (3):591-625
Bowen, R. 2001. Secretion of bile and the role of bile acids in digestion. Retrieved on June 27, 2011 from http://www.vivo.colostate.edu/hbooks/pathphys/digestion/liver/bile.html.
Boyd, C.E. 1990. Water Quality in Ponds for Aquaculture. Alabama Agriculture Experiment Station, Auburn University, Alabama, USA.
Brockmark, S.L., Bohlin, N.T., Bjornsson, B.T. and Johnson, J.L. 2007. Effect of rearing density and structural complexity on the pre and post release performance of Atlantic salmon. Transaction of the American Fisheries Society 136:1453-1462.
Burmeister, S.S., Kailasanath, V. and Fernald, R.D. 2007. Social dominance regulates androgen and estrogen receptor gene expression. Hormones and Behavior 51: 164 – 170.
Campbell, P.M., Pottinger, T.G. and Sumpter, J.P. 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120: 151–169.
Chakraborty, S.B. and Banerjee S. 2010. Effect of stocking density on monosex Nile tilapia growth during pond culture in India. World Academy of Science, Engineering and Technology 68:1511-1515.
Chandroo, K.P., Duncan, I.J.H. and Moccia, R.D. 2004. Can fish suffer?: perspectives or sentience, pain, fear and stress. Appl. Anim. Behav. Sci. 86: 225-250
Contreras-Sanchez, W.M., Schreck, C.B., Fitzpatrick, M.S. and Pereira, C.B. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biology and Reproduction 58: 439 – 447.
DiBattista, J.D., Levesque, H.M., Moon, T.W. and Gilmour, K.M. 2006. Growth depression in socially subordinate rainbow trout *Oncorhynchus mykiss*: more than a fasting effect. Physiol. Biochem. Zool. 79: 675-687.
Danting, M.J.C. 1992. Optimization of UV radiation treatment and cryopreservation of *O. niloticus* spermatozoa for induced gynogenesis. Unpublished Masteral thesis. Institute of Aquaculture, University of Stirling, Stirling Scotland. p. 74.
Davis, K.B. and McEntire, M.E. 2006. Effect of photoperiod on feeding, intraperitoneal fat, and IGF-I in sunshine bass. Annual Meeting World Aquaculture Society 37:431-436.

Dean, D.B., Whitlow, Z. and Borski R.J. 2003. Glucocorticoid receptor upregulation during seawater adaptation in a euryhaline teleost, the tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 132:112-118.

Degger, B., Upton, Z., Soole, K., Collet, C. and Richarson, N. 2000. Comparison of recombinant barramundi and human insulin-like growth factor (IGF)-I in juvenile Barramundi (*Lates calcarifer*): *In vivo* metabolic effects, association with circulating IGF-binding proteins, and tissue localization. Gen. Comp. Endocrinol. 117: 395-403.

Doyon, C., Gilmour, K.M., Trudeau, V.L. and Moon, T.W. 2003. Corticotropin-releasing factor and neuropeptide Y mRNA levels are elevated in the preoptic area of socially subordinate rainbow trout. General and Comparative Endocrinology, 133: 260-271.

Earley, R.L., Blumer, L.S. and Grober, M.S. 2004. The gall of subordination: changes in gall bladder function associated with social stress. Proc. R. Soc. Lond. 271: 7-13.

El-Azab, A.I., Adel-Ghafar, A.E., Nasr, M.T. and Issa, E.S. 1996. Effect of exogenous PGF2alpha and/or GnRH on libido and semen production of aged Friesian bulls. Journal of Veterninary Medicine 44 : 529-533.

Fox, H.E., White, S.A., Kao, M.H.F., and Fernald, R.D. 1997. Stress and dominance in a social fish. Journal of Neuroscience, 17: 6463-6469.

Gabor, G., Sasser, R.G., Kastelic, J.P., Coulter, G.H. Everson, D.O., Falkay, G., Meezes, M., Bozo, S., Cook, R.B., Volgyi, J.C., Barany, I. and Szaasz, F. 1998. Endocrine and thermal responses to GnRH treatment and prediction of sperm output and viability in Holstein-Friesian breeding bulls. Theriogenology 50: 177 – 183.

Gómez-Laplaza, L.M. and Morgan, E. 2003. Theinfluence of social rank in angelfish, *Pterophyllum scalare*, on locomotor and feeding activities in a novel environment. Laboratory Animals, 37: 108-120.

Goncalves-de-Freitas, E. 1999. Investimento reprodutivo e crescimento em machos de tilapia-do-Nilo. Tese de doutorado. Instituto de Biociencias, Universidade Estadual Paulista, Botucatu, SP.

Höglund, E., Kolm, N. and Winberg, S. 2001. Stress-induced changes in brain serotonergic activity, plasma cortisol and aggressive behavior in Arctic charr (*Salvelinus alpinus*) is counteracted by L-DOPA. Physiological Behavior, 74: 381-389.

Holfman, H.A. and Fernald, R.D. 2000. Social status controls somatostatin neuron size and growth. J Neurosci. 20: 4740-4744.

Jentoft, S., Topp, N., Seeliger, M., Malison, J.A., Barry, T.B., Held, J.A., Roberts S. and Goetz, F. 2005. Lack of growth enhancement by exogenous growth hormone treatment in yellow perch (*Perca flavescens*) in four separate experiments. Aquaculture, 250: 471–479.

Maeda, K. and Tsukamura, H. 2006. The impact of stress on reproduction: are glucocoticoids inhibitory or protective to gonadotropin secretion. Endocrinology 147: 1085-1086.

Montero, D., Izquierdo, M.S., Tort, L., Robaina L. and Vergara, J.M. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiology and Biochemistry 20:50-60.

Mostl, E. and Palme, R. 2002. Hormones as indicators of stress. Domestic Animal Endocrinology, 23:67-74.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. and Blokhuis, H.J. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23: 925-935.

Korzan, W.J., Ø. Øverli, and Summers, C.H. 2006. Future social rank: Forecasting status in the green anole (*Anolis carolinensis*). Acta Ethology, 9: 48-57.

Korzan, W.J., Robison, R.R., Zhao, S. and Fernand, R.D. 2008. Color change as potential behavioral strategy. Horm. Behav. 54: 463-470.

Kurokura, H. and Oo, K.M. 2008. Evaluation of fertilizing capacity of cryopreserved rainbow trout sperm. Fisheries Science 74: 621 – 626.

Little, D.C. and Bhujel, R.C. 2003. Advanced nursing of mixed-sex and mono-sex tilapia (*Oreochromis niloticus*) fry, and its impact on subsequent growth in fertilized ponds. Aquaculture 221:265–2.

Øverli, Ø., Winberg, S., Damsgard, B. and Jobling, M. 1998. Food intake and spontaneous swimming activity in Arctic charr (*Salvelinus alpinus*): role of brain serotonergic activity and social interactions. Canadian Journal of Zoology, 76: 1366-1370.

Øverli, Ø., Harris, C.A. and Winberg, S. 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationship on brain monomines and cortisol in rainbow trout. Brain Behavior and Evolution, 54: 263-275.

Øverli, Ø., Korzan, W.L., Höglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G.L., Barton, B.A., Øverli, E., Renner, K.J. and Summers, C.H. 2004. Stress coping style predicts aggression and social dominance in rainbow trout. Hormones and Behavior, 45: 235- 243.

Øverli, Ø., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers C.H. and Nilsson, G.E. 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioural, and cognitive traits in non-mammalian vertebrates.neuroscience and Biobehavioral Reviews, 31: 396-412.

Payne, A.I. and Collimson, R.I. 1983. A comparison of the biological characteristics of *Sarotherodon niloticus* (L.) with those of *S. aureus* (Steindachner) and other tilapia of the delta and lower Nile. Aquaculture 30: 335 – 351.

Petrauskiené, L. 1996. Effects of social stress in rainbow trout at different stocking densities. (www-heb:pac.dfo-mpo.gc.ca/congress/1996/Applied/Petrauskiene1.pdf.)

Picha, M.E., Silverstein, J.T. and Borski, R.J. 2006. Discordant regulation of hepatic IGF-I mRNA and circulating IGF-I during compensatory growth in a teleost, the hybrid striped bass (Morone chrysops X M. saxatilis). Gen. Comp. Endocrinol. 147:196-205.

Picha, M.E., Turano, M.J. Beckman, B.R. and Borski, R.J. 2008a. Hormonal biomarkers of growth and applications to aquaculture: A mini-review of growth hormone, insulin-like growth factor-I and IGF binding proteins as growth indicators in fish. NAJA 70: 196–211.

Picha, M.E., Turano, M.J., Tipsmark, C.K. and Borski, R.J. 2008b. Regulation of endocrine and paracrine sources of insulin-like growth factors and growth hormone receptor during compensatory growth in a teleost fish. J. Endocrinol. 199:81-94.

Porchas, M.M., Cordova, L.R.M. and Enriquez, R.R. 2009. Cortisol and glucose: reliable indicators of fish stress? Pan-American Journal of Aquatic Sciences, 4(2): 158-178.

Pottinger, T.G. and Carrick, T.R. 2001. Stress responsiveness affects dominant-subordinate relationships in rainbow trout. Hormones and Behavior, 40: 419-427.

Parikh, V.N., Clement, T.S. and Fernald, R.D. 2006. Androgen level and male social status in African cichlid, *Astatotilapia burtoni.* Behavioral Brain Research 166: 291 – 295.

Schreck, C.B. 2000. Accumulation and long-term effects of stress. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress: Assessment and Implications for Welfare. CAB International, Wallingford.

Siegfried, B., Frischknecht, H.R. and Waser, P.G. 1984. Defeat, learned submissiveness, and analgesia in mice: effects of genotype. Behaviour of Neural Biology, 421: 91-97.

Sloman, K.A. Gilmour, K.M., Taylor, A.C. and Metcalfe, N.B. 2000. Physiological effects of dominance hierarchies within groups of brown trout, Salmo trutta, held under simulated natural conditions. Fish Physiol. Biochem., 22:11-20

Sodeberg, R.W. and Meade, J.W. 1987. Effects of rearing density on growth survival and fin condition of Atlantic salmon. Prog. Fish-Cult. 49: 280-283.

Solati, N.H and Falahatkar, B. 2007. Stress responses in sub-yearling great sturgeon to the air exposure, Caspian Journal of Environmental Sciences 5:99-103.

Subasinghe, R.P. and Sommerville, C. 1992. Effects of temperature on the hatchability, development and growth of eggs and yolksac fry of *Oreochromis mossambicus* (Peters) under artificial incubation. Aquaculture and Fisheries Management 23: 31 – 39.

Vahdatpour, T., Nazer Adl, K., Nezhad, Y.E., Sis, N.M., Riyazi S.R. and Vahdatpour S. 2009. Effects corticosterone intake as stress-alternative hormone on broiler chickens: Performance and blood parameters. Asian Journal of Animal & Veterinary Advances, 4(1):16-21. Academic Journals Inc.

Vera Cruz, E.M., Bero, R.M.L., Bolivar, R.B. and Borski, R.J., 2009. Eye color predicts social dominance in Nile Tilapia *Oreochromis niloticus*. Paper presented at the WAS AQUA 2009, Seattle, Washington, USA. February 14-18, 2009.

Vera Cruz, E.M. and Brown, C.L. 2007. The influence of social status on the rate of growth, eye color pattern and insulin-like growth factor-I gene expression in Nile tilapia, *Oreochromis niloticus.* Horm. and Behav. 51: 611-619.

Vera Cruz, E.M., Brown, C.L., Luckenbach, J.A., Picha, M.E., Bolivar, R.B. and Borski, R.J. 2006. Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, *Oreochromis niloticus*. Aquaculture 251: 585-595.

Volpato, G.L., Luchiari, A.C., Duarte, C.R.A., Barreto, R.E. and Ramanzini, G.C. 2003. Eye color as an indicator of social rank in the fish Nile tilapia. Braz. J. Med. Biol. Res. 36: 1659-1663.

Winberg, S., Nilsson, G.E. and Olsén, K.H. 1992. Changes in brain serotonergic activity during hierarchic behavior in artic charr (*Salvelinus alpinus* L.) are socially induced. Journal of Comparative Physiology, 170, 93-99.

Winberg, S., Carter, C.G., McCarthy, I.D., He, Z.-Y., Nilsson G.E. and Houlihan, D.F. 1993. Feeding rank and brain serotonergic activity in rainbow trout *Oncorhynchus mykiss*. Journal of Experimental Biology, 179: 197-211.

Wood, A.W., Duan, C. and Bern, H.A. 2005. Insulin-like growth factor signaling in fish. International Review of Cytology, 243:215-85.

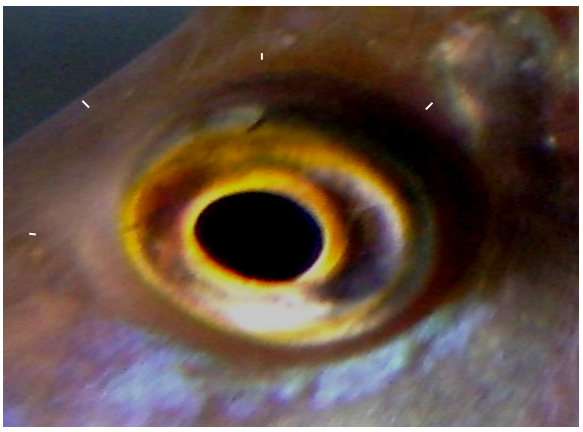


Figure 1. Eye color pattern of Nile tilapia.

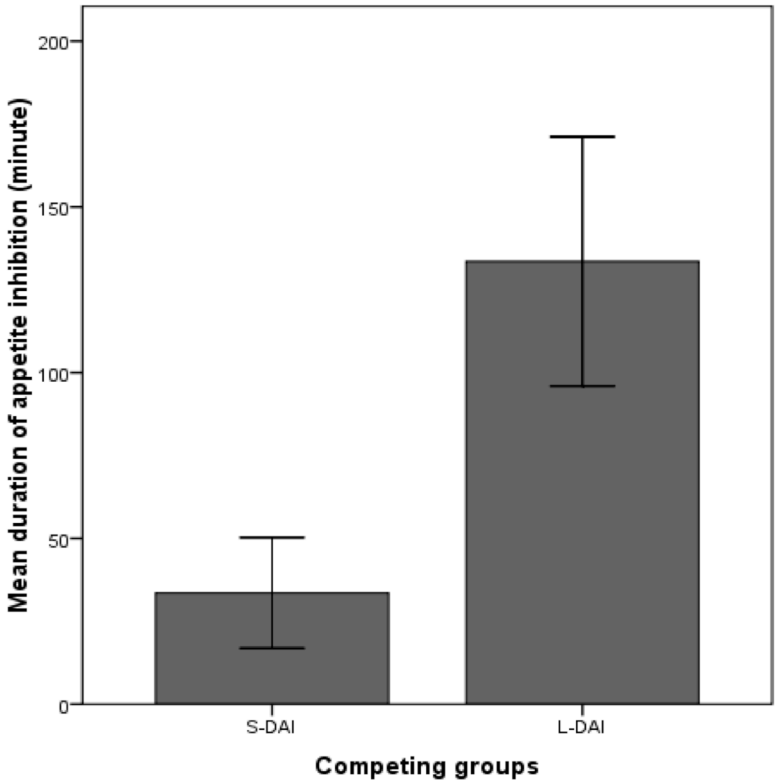


Figure 2. Average duration of appetite inhibition (minute) of the two competing groups. S-DAI, short DAI group; L-DAI, long DAI group. Average DAI were significantly different at $P < 0.01$. Mean ± SEM

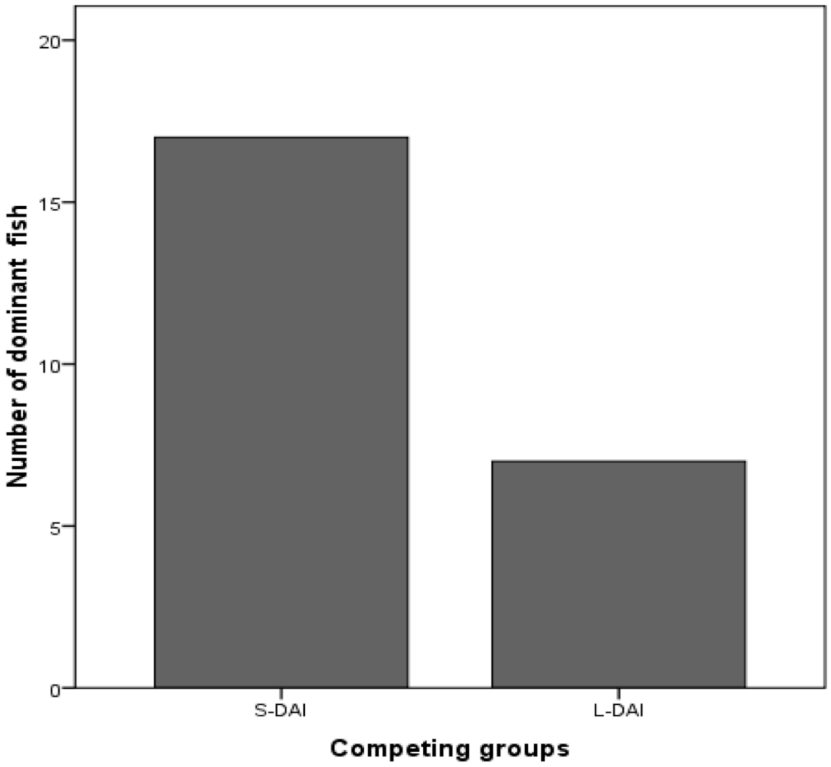


Figure 3. Number of dominant fish in the two competing groups. S-DAI: short DAI group; L-DAI: long DAI group. Frequency difference was significantly different at $P < 0.05$.

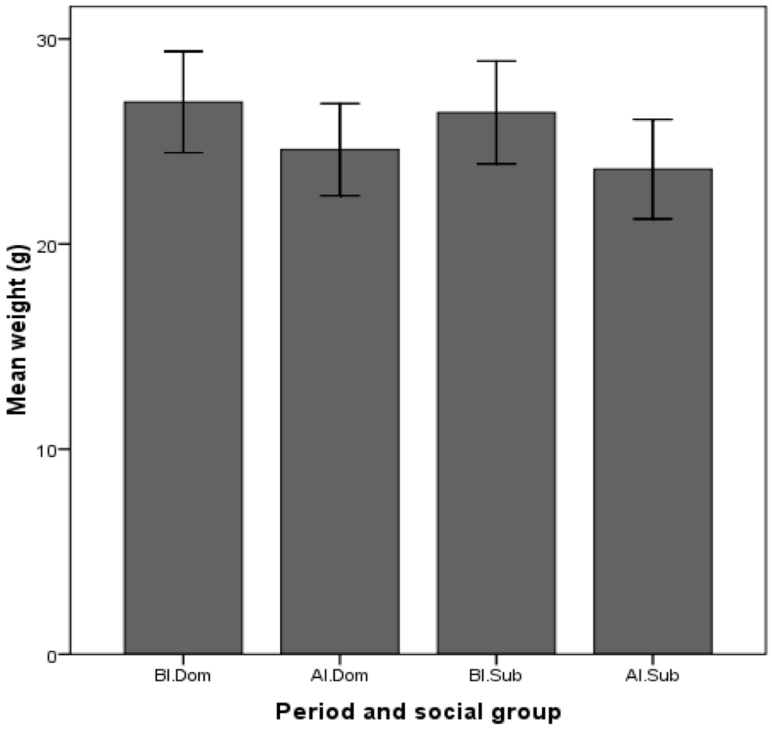


Figure 4. Mean weight (± SEM) of dominant and subordinate fish before and after the social interaction. BI.Dom: dominant - before the interaction; AI.Dom: dominant – after the interaction; BI.Sub: Subordinate – before the interaction; AI.Sub: Subordinate - after the interaction.

Table 1. Summary of time before first acceptance of food and feeding latency score (*i.e.* feeding response score) during the isolation period, and weight of competing individuals before the interaction and 1, 7 and 14 days after the first interaction.

Fish Code No.	Initial Weight (g)		Time before first food acceptance (hr)		Latency score		Weight one day after first interaction (g)		Weight seven days after first interaction (g)		Weight 14 days after first interaction (g)	
14 vs 1	24.4	23.9	2.39	3.51	21	22	25.1	23.4	30.5	25.5	35.1	28.0
3 vs 15	23.1	23.1	0.73	1.74	25	23	25.5	22.9	29.4	25.5	31.3	27.3
5 vs 18	25.1	25.0	2.47	3.44	23	23	25.3	24.2	26.2	27.4	30.7	28.3
9 vs 7	19.9	20.1	1.70	5.00	23	13	21.8	22.1	28.1	25.1	29.6	27.2
32 vs 8	22.6	21.5	0.16	3.73	21	19	23.1	20.5	25.9	23.1	28.5	24.9
11 vs 6	28.4	29.6	1.47	6.80	22	22	28.8	30.5	31.9	31.6	33.9	33.5
13 vs 4	24,.8	24.6	2.60	3.19	20	24	25.4	25.0	29.3	30.4	32.2	33.0
16 vs 2	28.0	27.6	0.34	8.19	22	22	28.3	26.5	38.3	30.0	40.2	30.7
45 vs 17	22.0	23.4	0.13	2.37	23	23	22.0	23.1	24.6	28.0	27.5	31.2
10 vs 12	26.2	26.4	2.02	2.98	27	20	27.2	26.8	32.5	31.5	33.9	
59 vs 46	23.3	23.2	0.04	0.18	23	24	23.2	23.6	26.7	24.8	30.8	28.8
33 vs 25	21.9	21.3	0.22	3.96	22	13	21.7	21.5	23.6	24.7	25.9	26.8
38 vs 23	20.0	20.4	0.18	1.40	21	19	19.4	20.5	22.6	24.4	25.9	27.0
40 vs 47	21.8	21.6	0.11	1.20	22	22	21.3	21.2	22.3	23.0	24.9	25.2
29 vs 48	25.8	26.3	0.17	0.65	25	25	26.4	25.8	29.2	30.5	31.9	33.1
37 vs 34	22.8	23.1	0.16	0.24	19	20	24.4	22.8	27.2	23.9	32.8	25.6
26 vs 35	25.5	25.4	0.07	3.71	23	14	25.8	25.1	27.4	27.2	29.5	30.8
24 vs 27	24.7	24.7	0.09	0.20	22	20	26.7	23.6		27.9		31.1
28 vs 36	24.0	24.1	0.02	0.24	27	17	23.3	23.0	26.5	27.6	28.5	33.0
49 vs 22	31.6	30.5	0.22	0.30	21	21	32.3	32.3	34.7	34.7	36.8	35.4
53 vs 21	28.1	28.2	0.13	3.10	14	23	27.1	28.7	30.0	31.6	31.8	35.1
43 vs 31	25.2	25.4	0.22	1.14	20	19	25.1	25.7	27.4	28.8	29.9	30.2
50 vs 30	25.5	25.5	0.02	0.41	19	20	25.1	26.0	27.0	28.6	29.4	30.8
44 vs 19	28.3	29.3	0.19	0.74	18	19	29.9	28.4	35.0	32.1	40.2	34.3
58 vs 39	26.5	20.9	0.06	0.30	24	20	26.4	21.0	28.8	23.6	31.6	27.0
57 vs 54	24.9	24.8	0.03	0.85	25	17	24.1	23.8	26.3		28.6	
51 vs 41	22.2	22.4	0.09	3.12	25	23	21.5	22.5	23.2	23.5	26.4	26.5
42 vs 56	27.7	27.2	0.10	0.30	22	18	27.0	26.4	28.3	27.0	30.3	29.5
52 vs 55	24.3	24.6	0.18	0.30	28	13	23.7	23.7	25.1	25.1	25.5	28.7

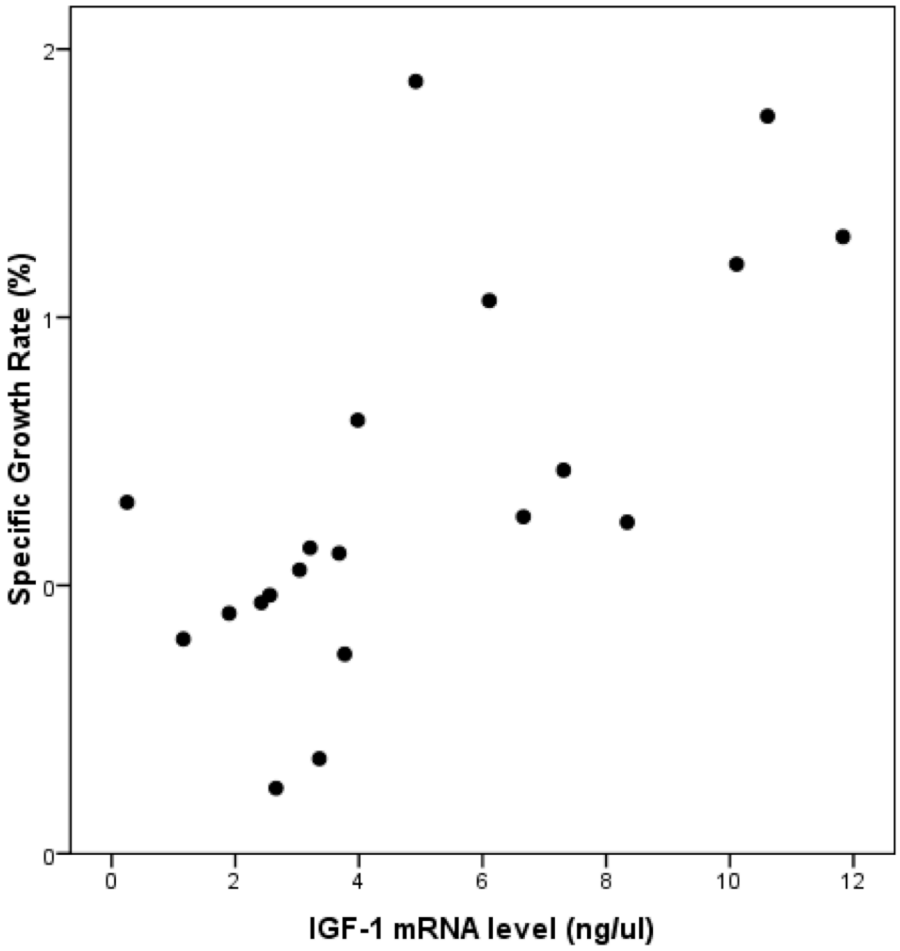


Figure 5. Correlation between IGF-I mRNA level (ng/µl) and specific growth rate of the dominant and subordinate fish, n = 20, $r = 0.60$, $P < 0.01$

Table 2. Average size of the eggs collected in each treatment. LSR, low stress response group; LHR, high stress response group

TREATMENT (SOCIAL GROUP)	AVERAGE SIZE OF THE EGGS (mm)			MEAN± SD [1, 2]
	REPLICATION			
	1	2	3	
LSRHSR♂♀	2.44	2.42	2.52	2.46[b] ± 0.0529
LSR♂♀	2.91	2.79	2.87	2.86[a] ± 0.0611
HSR♂♀		2.16		2.16[c]
LSR♂HSR♀	2.31	2.29	2.23	2.21[c] ± 0.0862
HSR♂LSR♀	2.62	2.54	2.50	2.55[b]± 0.0611

[1]Analysis based on unequal number of replications
[2]Means with similar superscript letters are not significantly different at 5 % level of significance by LSD

Table 3. Sperm motility scoring for the respective accumulated feeding response score by the male breeders.

FEEDING RESPONSE SCORE	MOTILITY SCORE
0	3
1	5
2	4
5	5
6	10
8	10
10	10
11	10
12	10
14	10
15	10
18	10

Table 4. Sperm density (sperm per ml) of the breeders under the accumulated feeding response score

FEEDING RESPONSE SCORE	SPERM DENSITY (SPERM PER ML)
0	$2.025 \times 10^{9(i)}$
1	$3.100 \times 10^{9(h)}$
2	$3.125 \times 10^{9(h)}$
5	$3.750 \times 10^{9(g)}$
6	$4.200 \times 10^{9(feg)}$
8	$4.075 \times 10^{9(fg)}$
10	$4.375 \times 10^{9(fe)}$
11	$4.725 \times 10^{9(de)}$
12	$5.100 \times 10^{9(cd)}$
14	$5.425 \times 10^{9(c)}$
15	$9.400 \times 10^{9(b)}$
18	$1.063 \times 10^{10(a)}$

Means with similar superscript letters are not significantly different at 5 % level of significance CV = 49.62%

Table 5. Spawning success during the 2nd production cycle.

TREATMENT (SOCIAL GROUP)	SPAWNING SUCCESS DURING THE 2ND PRODUCTION CYCLE			MEAN ± SD[1, 2]
	REPLICATION			
	1	2	3	
LSRHSR♂♀	33.33	50.00	66.67	50.00[a] ± 16.67
LSR♂♀	33.33	16.67	33.33	27.78[a] ± 9.62
HSR♂♀	0	0	0	0.00[b] ± 0.00
LSR♂HSR♀	66.67	33.33	50.00	50.00[a] ± 16.67
HSR♂LSR♀	33.33	33.33	16.67	27.78[a] ± 9.62

[1] Based on arcsine square root percentage transformation
[2] Means with similar superscript letters are not significantly different at 5% level of significance

Table 6. Average eggs produced per female at one spawning.

TREATMENT (SOCIAL GROUP)	AVERAGE EGGS PRODUCED PER FEMALE			MEAN ± SD[1, 2]
	REPLICATION			
	1	2	3	
LSRHSR♂♀	711	597	608	638.67[bc] ± 62.88
LSR♂♀	813	1113	929	951.67[a] ± 151.28
HSR♂♀	0	0	0	0.00[d]
LSR♂HSR♀	632	417.5	436	495.17[c] ±118.86
HSR♂LSR♀	730	946	715	797.00[ab] ± 129.26

[1] Analysis based on log (x+1) transformation
[2] Means with similar superscript letters are not significantly different at 5% level of significance

Table 7. Total number of eggs produced in the 1st and 2nd production cycles.

TREATMENT (SOCIAL GROUP)	NUMBER OF EGGS PRODUCED DURING THE 1ST AND 2ND CYCLES							
	1ST PRODUCTION[1]			MEAN[3] (± SD)	2ND PRODUCTION[2]			MEAN[3] (± SD)
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	2551	2421	4809	3260.33[a] (± 1342.76)	1423	1793	2435	1883.67[a] (± 512.06)
LSR♂♀	3290	2016	2147	2484.33[a] (± 700.80)	1626	1113	1858	1532.33[a] (± 381.23)
HSR♂♀		2142		2142.00[a]	0	0	0	0.00[b]
LSR♂HSR♀	609	704	1289	867.33[b] (± 368.25)	2528	835	1309	1557.33[a] (± 873.39)
HSR♂LSR♀	1073	506	873	817.33[b] (± 287.57)	1459	1892	715	1355.33[a] (± 595.31)

[1] Analysis based on unequal number of replications
[2] Analysis based on log (x+1) transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 8. Total egg production in the two production cycles.

TREATMENT (SOCIAL GROUP)	TOTAL EGG PRODUCTION		
	1ST PRODUCTION	2ND PRODUCTION	TOTAL EGG PRODUCED
	MEAN ± SD	MEAN ± SD	
LSRHSR♂♀	3260.33[a] ± 1342.76	1883.67[a] ± 512.06	5144.00[a]
LSR♂♀	2484.33[a] ± 700.80	1532.33[a] ± 381.23	4016.66[ab]
HSR♂♀	2142.00[a]	0.00[b]	2142.00[c]
LSR♂HSR♀	867.33[b] ± 368.25	1557.33[a] ± 873.39	2424.66[bc]
HSR♂LSR♀	817.33[b] ± 287.57	1355.33[a] ± 595.31	2172.66[bc]

Means with similar superscript letters are not significantly different at 5% level of significance

Table 9. Hatching rate of the collected eggs over two production cycles.

TREATMENT (SOCIAL GROUP)	HATCHING RATE IN TWO CYCLES (%)							
	1ST PRODUCTION[1]			MEAN ± SD	2ND PRODUCTION[2]			MEAN ± SD[3]
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	83.73	82.94	68.12	78.26[a] ± 8.79	87.7	86.11	76.88	83.56[a] ± 5.84
LSR♂♀	93.50	93.70	90.13	92.44[a] ± 2.01	84.56	81.49	86.33	84.13[a] ± 2.45
HSR♂♀	0.00	76.00	0.00	76.00[a]	0	0.00	0.00	0.00[b] ± 0.00
LSR♂HSR♀	80.62	88.78	93.64	87.68[a] ± 6.58	85.88	90.18	77.23	84.43[a] ± 6.60
HSR♂LSR♀	87.51	86.76	69.53	81.27[a] ± 10.17	78.48	79.33	84.34	80.72[a] ± 3.17

[1] Analysis based on unequal number of replications
[2] Analysis based on arcsine square root percentage transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 10. Survival rate of the post-fry after three weeks.

TREATMENT (SOCIAL GROUP)	SURVIVAL RATE (%) IN TWO CYCLES							
	1ST PRODUCTION[1]			MEAN[3/] (± SD)	2ND PRODUCTION[2]			MEAN[3] (± SD)
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	71.15	75.34	49.32	65.27[a] (± 13.97)	72.17	81.82	61.93	72.00[a] (± 9.95)
LSR♂♀	75.93	87.50	82.07	81.83[a] (± 5.79)	72.88	75.56	80.52	78.14[a] (± 3.88)
HSR♂♀		47.53		47.53[b]	0	0	0	00.00[c] (± 0.00)
LSR♂HSR♀	55.5	68.47	69.98	64.65[a] (± 7.96)	64.44	78.8	63.41	68.26[ab] (± 8.60)
HSR♂LSR♀	68.59	55.53	52.58	58.90[b] (± 8.52)	58.67	59.73	55.52	57.97[b] (± 2.19)

[1] Analysis based on unequal number of replications
[2] Analysis based on arcsine square root percentage transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 11. Length of the post-fry after three weeks rearing period

LENGTH OF THE POST-FRY AFTER 3 WEEKS (mm)								
TREATMENT (SOCIAL GROUP)	1st PRODUCTION[1]			MEAN ± SD[3]	2nd PRODUCTION[2]			MEAN ± SD[3]
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	18.57	19.01	19.37	18.98^{b} (± 0.40)	18.61	18.17	18.33	18.37^{c} (± 0.22)
LSR♂♀	21.18	20.88	20.66	20.91^{a} (± 0.26)	20.67	19.79	20.56	20.34^{a} (± 0.48)
HSR♂♀	0.00	13.88	0.00	13.88^{c}	0.00	0.00	0.00	0.00^{d} (± 0.00)
LSR♂HSR♀	18.01	18.81	18.14	18.32^{b} (± 0.43)	18.56	18.10	18.22	18.29^{c} (± 0.24)
HSR♂LSR♀	19.68	19.31	19.14	19.38^{b} (± 0.28)	19.49	19.24	19.00	19.24^{b} (± 0.25)

[1] Analysis based on unequal number of replications
[2] Analysis based on arcsine square root percentage transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 12. Weight of the post fry after three weeks rearing period.

WEIGHT OF THE POST FRY AFTER 3 WEEKS (g)								
TREATMENT (SOCIAL GROUP)	1st PRODUCTION[1]			MEAN ± SD[3]	2nd PRODUCTION[2]			MEAN ± SD[3]
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	0.26	0.26	0.27	0.26^{b} ± 0.0058	0.24	0.23	0.25	0.24^{c} ± 0.0100
LSR♂♀	0.33	0.30	0.30	0.31^{a} ± 0.0173	0.32	0.29	0.30	0.30^{a} ± 0.0153
HSR♂♀	0.00	0.11	0.00	0.11^{d}	0.00	0.00	0.00	0.00^{d} ± 0.0000
LSR♂HSR♀	0.23	0.24	0.24	0.24^{c} ± 0.0058	0.24	0.23	0.23	0.23^{c} ± 0.0058
HSR♂LSR♀	0.29	0.28	0.28	0.28^{b} ± 0.0058	0.28	0.27	0.27	0.27^{b} ± 0.0058

[1] Analysis based on unequal number of replications
[2] Analysis based on log (x+1) transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

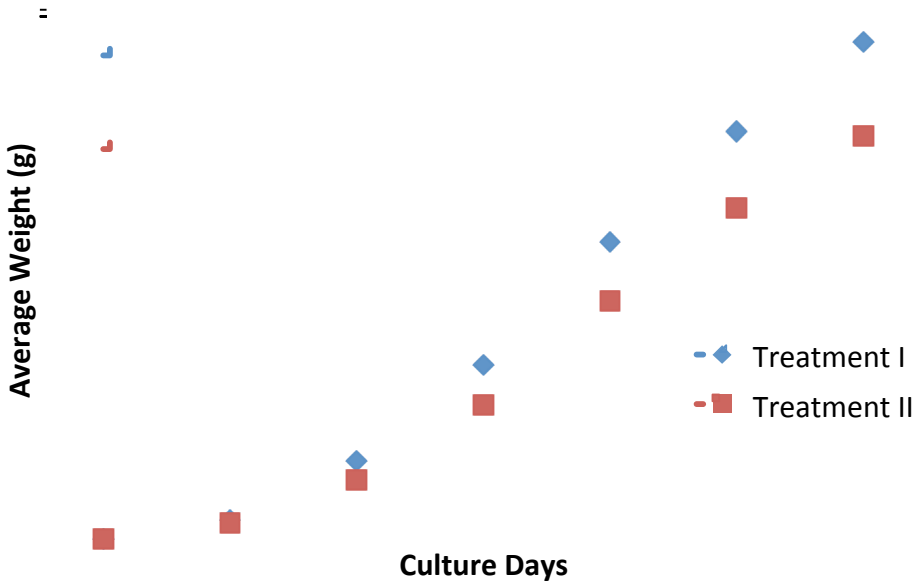


Figure 6. Average weight of Nile tilapia during 90 days of culture in ponds. Treatment I – fingerlings derived from low stress response broodfish; Treatment II - fingerlings derived from high stress response broodfish.

Table 13. Growth performance of Nile tilapia produced from broodstock with low and high stress responses.

Parameters	**Treatments**	
	Sex-reversed fingerlings produced from Low Stress Response Broodfish (Mean ± SEM)	**Sex-reversed fingerlings produced from High Stress Response Broodfish** (Mean ± SEM)
Initial weight (g)	0.199 ± 0.10	0.212 ± 0.20
Final average weight (g)	111.27 ± 19.20	90.31 ± 24.10
Initial length (cm)	2.93 ± 1.20	2.90 ± 2.00
Final average length (cm)	17.16 ± 0.77	17.44 ± 2.60
Gain in weight (g)	111.07 ± 19.10	90.10 ± 23.90
Daily gain in weight (g)	1.23 ± 0.20	1.00 ± 0.30
Gain in length (cm)	14.2 ± 0.80	14.5 ± 0.70
Daily gain in length (cm)	0.158 ± 0.01	0.162 ± 0.01
Feed Conversion Ratio	1.4 ± 0.08	1.7 ± 0.23
Specific Growth Rate (%)	5.51 ± 0.33	5.66 ± 0.55
Yield per hectare (kg ha^{-1})	1596.7 ± 219.0	1221.7 ± 407.0
Feed consumed per hectare (kg ha^{-1})	2205.3 ± 326.30	1890.2 ± 371.50
Survival (%)	73.0 ± 3.90	72.3 ± 5.20

Table 14. Average minimum and maximum readings for water quality parameters during the culture period.

Parameters	Sex-reversed fingerlings produced from Low Stress Response Broodfish		Sex-reversed fingerlings produced from High Stress Response Broodfish	
	Min	Max	Min	Max
Dissolved Oxygen (mg-L^{-1})	2.4	4.2	1.8	4.1
Water Temperature (^{o}C)	28.2	30.1	28.1	30.0
Hydrogen-Ion (pH)	7.3	9.0	7.3	9.1
Total Ammonia Nitrogen (mg L^{-1})	0.051	0.162	0.054	0.190
Secchi Disc Visibility (cm)	20	60	21.7	56.7
Alkalinity (mg L^{-1})	107.7	159.0	101.0	151.3
Phosphorus (mg L^{-1})	0.092	0.237	0.083	0.247

Table 15. Body weight and survival of Nile tilapia reared at different stocking densities in hapas.

Treatment	Stocking Density (pcs)	nitial Weight g)	Final Weight (g)	al
1	250	$7.30^{a} \pm 1.69$	$8.13^{x} \pm 2.05$	$87.6^{a} \pm 9.59$
2	250	$4.15^{b} \pm 0.16$	$6.75^{xy} \pm 0.33$	$61.10^{b} \pm 15.49$
3	500	$4.87^{b} \pm 0.77$	$4.94^{z} \pm 0.25$	$70.05^{b} \pm 4.37$
4	1000	$4.87^{b} \pm 0.77$	$4.66^{z} \pm 0.18$	$67.00^{b} \pm 4.61$

Values represent mean ± standard deviation. 1 (control) - Low density, mixed-sex; 2 - Low density, sex-reversed; 3 - Medium density, sex-reversed; and 4 - High density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 16. Specific growth rates (%) of Nile tilapia reared at different stocking densities during the course of the study

Treatment	Day 0-10	Day 10-24	Day 24-30	Day 0-30
1	-0.039 ± 1.14	1.047[ab] ± 0.91	-0.631 ± 7.98	0.349 ± 1.37
2	1.449 ± 3.03	2.344[a] ± 0.91	0.299 ± 1.75	1.637 ± 1.52
3	0.158 ± 0.92	0.503[b] ± 0.72	-0.135 ± 1.52	0.260 ± 0.27
4	-0.473 ± 1.55	-0.084[b] ± 0.33	0.382 ± 0.53	-0.120 ± 0.53

Values represent percentage mean ± standard deviation on the specific growth rates of Nile tilapia reared at different stocking densities. 1 (control) - Low density, mixed-sex; 2 - Low density, sex-reversed; 3 - Medium density, sex-reversed; and 4 - High density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 17. Mean IGF-1 mRNA concentration (ng/µl) of Nile tilapia after 7 days of rearing at different stocking densities.

Treatment Density Group	IGF-1 mRNA (ng/µl)
1	27.03[ab] ±5.24
2	31.59[a] ±6.94
3	14.44[b] ±10.90
4	7.79[b] ±7.64

GF-1 mRNA levels (ng cDNA/µl total RNA) values are shown as mean ± standard deviation. Treatment groups: 1 (control) – low density, mixed-sex; 2 - low density, sex-reversed; 3 - medium density, sex-reversed; and 4 - high density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 18. The hepatosomatic and cholecystic indices of Nile tilapia reared in different stocking densities.

Treatment Density	Liver Size and Hepatosomatic Index			Gall Bladder Size and Cholecystic Index		
	Weight (g)	Length (mm)	HSI (%)	Weight (g)	Length (mm)	CI (%)
1	0.20[a]±0.02	3.55[a]±0.49	1.09[a]±0.31	0.05[a] ±0.01	0.96[a]±0.43	26.4[a]±17.39
2	0.11[b]±0.02	2.17[b]±0.45	1.11[a]±0.20	0.04[ab]±0.01	0.90[a]±0.23	31.5[a]±23.22
3	0.08[c]±0.01	1.70[b]±0.28	0.96[a] ±0.22	0.02[b]±0.004	0.67[a]±0.08	33.37[a]±21.23
4	0.07[c]±0.01	2.07[b]±0.45	0.99[a] ±0.07	0.03[b]±0.004	0.68[a]±0.05	41.3[a]±17.42

Hepatosomal and cholecystic parameters shown as mean ± standard deviation. 1 (control) - low density, mixed-sex; 2 - low density, sex-reversed; 3 - medium density, sex-reversed; and 4 - high density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 19. Mean blood glucose levels of Nile tilapia reared at different stocking densities

Treatment Density	Blood Glucose (mg/dl)
1	$81.67^{ab} \pm 11.05$
2	$60.67^{bc} \pm 16.01$
3	$94.50^{a} \pm 19.01$
4	$45.75^{c} \pm 15.09$

Blood glucose values are expressed as mean ± standard deviation. 1 (control) - low density, mixed-sex; 2 - low density, sex- reversed; 3 - medium density, sex-reversed; and 4 - high density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 20. Total red blood cell and white blood cell counts (x 10^9 cells per ml of blood) of Nile tilapia reared at different stocking densities.

Treatment Density	RBC	WBC
1	3.7^{a}	1.576^{a}
2	2.85^{b}	1.573^{a}
3	2.45^{b}	1.125^{b}
4	2.0^{c}	0.496^{c}

Treatment groups: 1 (control) - low density mixed-sexed; 2 - low density sex-reversed; 3 - medium density, sex-reversed; and 4 - high density sex-reversed. Values with the same letter superscripts are not significantly different.

Sustainable Integrated Tilapia Aquaculture: Aquaponics and Evaluation of Fingerling Quality in Tabasco, Mexico

Quality Seedstock Development/Experiment/09QSD02UA

Wilfrido Contreras-Sanchez, Mario Fernández-Perez, Carlos A. Alvarez-González,
Arlette Hernández Franyutti, Ulises Hernández-Vidal, and Alejandro Mcdonal-Vera
Universidad Juárez Autónoma de Tabasco
Villahermosa, México

Kevin Fitzsimmons
University of Arizona
Tucson, Arizona, USA

ABSTRACT

Integrated aquaculture and agriculture can provide a more sustainable production system in virtually any instance where plants are to be irrigated. In situations where resources are limited, provision of fresh fish and nutrient rich effluent to irrigate and fertilize vegetables can be even more important. Likewise, high quality fingerlings are required for farmers to be successful in their cultivation efforts. Having an unbiased evaluator of fingerling quality is something that is best done by a university of other entity with the resources and no particular interest in any one source or strain. In this two part investigation we collaborated with two indigenous communities to install and begin operation of integrated tilapia and vegetable plots, initially with Nile tilapia and habanero peppers. A third system was constructed on the campus of the Universidad Juarez Autonomo de Tabasco in Villahermosa, Mexico for training of students and to share with members of the indigenous communities when they were brought to the campus for workshops.

The first indigenous demonstration site at Caridad Guerrero was destroyed in a flood and had to be rebuilt. But after that, the projects have proven successful. The fish have grown quickly from fry to over 60 g and the peppers have begun to yield edible produce. The peppers had a limited value in the local community. However by assisting the community to pickle the peppers and sell in jars greatly increased the value and allowed the product to be sold to larger communities. A simple enterprise budget was prepared and demonstrated that the integrated system as built should have a pay-back period of 2.05 years.

The evaluation of fry is still underway. However, of the seven strains, four were found with parasitic loads that would reduce growth. All seven were found to contain one or more opportunistic bacterial infections that would likely decrease growth if the fish were stressed.

INTRODUCTION

Conservation and multiple use of water has become an important practice, even in parts of the world with large natural water resources. However, adopting water conservation principals sometimes requires a strong cultural education component. In this regard, multiple institutions must cooperate with the common goal of sharing information, ideas, and local cultural practices in which to develop water re-use practices. The sharing of cultural knowledge and development of best management practices is necessary to establish community support as well as monitoring protocols needed for

long-term success. Water conservation efforts are readily adopted, especially in developing and at risk regions of the world, because of the demonstrated economic and environmental benefits. One area in which a greater level of cooperation and social-economic development can be achieved through water re-use efforts is in integrated aquaculture.

Integrated aquaculture/agriculture systems are increasingly being promoted as an environmentally sustainable method for producing aquatic and terrestrial crops. The main goal of integrated aqua-agro systems is to improve nutrient cycling and energy flow in the system to obtain maximum benefits in the production of food and fiber (Chan, 1993). In integrated systems, wastes from one system component are recycled as inputs to another system component. Through waste recycling, the use of pond sediment organic matter as a crop fertilizer, and of pond water for irrigation, establishes linkages between aquaculture ponds and crops.

The treatment and discharge of aquaculture effluent and resulting negative impacts on the environment remains a critical issue that is threatening the sustainable growth of the aquaculture industry. Even the discharge of effluent that has been treated to levels acceptable under limitation guidelines (ELGs) (EPA, 2002) through the use of dilutions, may pose long-term environmental risks. The resulting negative impacts will continue to intensify in the future if sustainable practices are not developed in commercial scale. A new model (Integrated Aquaculture/Agriculture System model or IAAS) has been developed in order to face the above issues reusing organic matter and nitrogen dynamics through aquaculture and integrated conventional agriculture. Integrated aquaculture/agriculture systems can be used to treat aquaculture effluents, increase farm productivity through efficient resource utilization, spread financial risk through diversification and reduce system nutrient losses (Singh et al., 1996; Williams, 1997).

The effluents from aquaculture have been shown to contain the necessary ratios and amounts of nutrients for many crop plants (Rakocy et al. 1993). Thus, significant economic and environmental benefits can be achieved by linking aquaculture and crop irrigation, as the environmental pollution from aquaculture is converted to a valuable source of fertilizers. This translates to a value-added process by which the nutrient laden effluents are converted to profitable plant biomass. A specific case has been identified for the proposed project with which to demonstrate the concepts of implementing water re-use concepts in indigenous local communities through the cooperation of multiple institutions and rural farmers. The Universidad Juarez Autonoma de Tabasco (UJAT) has already developed a partnership in aquaculture with several indigenous communities during the Condor-Eagle Project jointly supported by the Aquaculture CRSP and Heifer International. The proposed project will augment past efforts towards community based aquaculture, with the development and implementation of integrated agriculture-aquaculture practices. This project will provide a unique outlook on how water re-use in aquaculture-agriculture systems facilitate social-economic development. Determining the degree to which the system improves household income is an especially important aspect of adoption.

Fingerling quality has become a significant concern among tilapia farmers in Southeastern Mexico during recent years. The problem goes to the basics, since several fingerling vendors are introducing fish at a lower price; however, there is no evidence that farmers are buying good quality fish, nor is there evidence of the effectiveness of the masculinization treatment used. Members of the Association of Tilapia Producers of Tabasco have expressed their concern to the personnel of the Tropical Aquaculture Laboratory (UJAT) regarding bad quality fingerlings. This low-quality product is mainly perceived as low growth and low survival. There are also concerns that the "purity" of the line sold is not trustworthy. Some fingerling retailers assure they are selling "GIFT", "YY males", "Chitralada", or Rocky Mountain" strains besides the local lines produced either by the State government ("Tabasco" line -supported through two consecutive A-CRSP projects-) or some private hatcheries ("Stirling").

In Latin America, broodstock and seed supply have been identified as one of the major constraints to production increases. In the 2001 expert panel meeting organized by the PD/A CRSP, inadequate availability and quality of fry (and broodstock) were listed as a researchable priority. Part of the problem was solved by supporting a line selection program that allowed the formation of the "Tabasco line" that supports the fry production in the State Hatchery "Mariano Matamoros". This is still an ongoing project supported mainly by the Tabasco Government and UJAT. However, the production of this hatchery is primarily used for restocking lagoons or ponds were the farmers do not require single sex populations (the government does not produce masculinized fingerlings). Some private farms have acquired the "Tabasco line" and they sell the masculinized fish. In the region, tilapia culture has become the principal aquacultural activity. Unfortunately, the introduction of different lineages of unknown origin and the lack of growth performance information has created disappointment and uncertainty. It is important to define a strategy to produce reliable information despite the origin of the fish. It is possible that the strains are originals, but the environmental conditions under which they were created may not favor performance under climatic conditions for Central America.

To help farmers in Southeastern Mexico solve these speculations, we propose to conduct an objective, unbiased experiment to contrast growth performance, time to reach market size (300 gr), survival, total biomass and cost of production. With this information in hand, farmers will have information to base their decisions and purchase the fingerlings based either in cost of production or growth performance.

OBJECTIVES

1. To build three demonstration aquaculture – agriculture units in indigenous communities.
2. To evaluate the success of local farmers adopting multi-use concepts to grow fish and plant crops.
3. To provide an enterprise model documenting the cost – benefits of the integrated system.
4. To compare at least five different tilapia strains used in Southeastern Mexico.
5. To provide a protocol for tilapia strain evaluation based on growth and economic variables.
6. To provide objective information for farmers to help decide which strains produce best results.

METHODS AND MATERIALS

Building three demonstration aquaculture – agriculture units in indigenous communities

We built two integrated systems at indigenous communities. There was a third indigenous site selected but this site was impacted with severe flooding, causing several damages at the community level and at the project level, these flooding events were caused by the modification the riverbeds, we had the most severe raining season in 30 years, these condition remained all year around. Besides the adverse condition at the selected site the access routes had severe damages, which hampered the access to the site. Due to all these causes, we decided to built the third system at UJAT, where is a high zone free of flooding and it is only 30 km away from the selected site, building the third system at UJAT increased the impact range through demonstration and training, UJAT site has been used as a training site for farmers and educational site for students.

Demonstration and Evaluation of an Integrated Aquaculture – Agriculture System for Indigenous Farmers in Tabasco, Mexico

To build three demonstration aquaculture – agriculture units in indigenous communities

Two workshops were held at UJAT facilities the first workshop was given by Dr. Kevin Fitzsimmons, Dr. Dennis McIntosh and Dr. Rafael Martinez-Garcia on Integrated aquaculture agriculture systems. The second workshop was given by Tracy Holstein on the use of biofloc systems.

Three integrated aquaculture agriculture systems in two indigenous communities and one educational and demonstration site were built. An integrated aquaculture agriculture system was developed in an indigenous Chol community at Caridad Guerrero, Tacotalpa county in Tabasco. The effluents of 1500 Tilapia, which were fed 2 twice per day at a rate of 5% biomass of Tilapia feed, contained in a 12 m^3 geomembrane tank were used to irrigate habanero peppers twice per day. The peppers were grown from seedlings in three agricultural beds (10 x 15m), constructed with a 3% slope to capture effluents for analysis. Sampling of Tilapia and habanero were made each month, total length and weight were taken for the Tilapia and length (height) for habanero pepper. Total product harvest was accomplished by weighing the total production (fruit) of each plant.

Assembling geomembrane tank for fish

Preparing aquaponics growing bed

The second site was developed at a Chontal indigenous community in Oxiacaque, Nacajuca county. Except for the agricultural unit measures (5 x 10m) most of the procedure was conducted as described for Caridad Guerrero.

The third site an educational and demonstrative system was built at UJAT, following the procedure as was conducted in Caridad Guerrero.

Tilapia and habanero seedlings were transferred to the sites at different times. Data for biomass crop production was analyzed in order to obtain mean values for each case. Analysis of water quality was performed monthly, only for the Caridad Guerrero system measuring nitrites, nitrates and ammonia in order to calculate total Nitrogen.

Evaluation of the success of local farmers adopting multi-use concepts to grow fish and plant crops.

The people performed a partial harvest of habanero peppers at the Caridad Guerrero site. Due to the delay suffered by flooding events the aquaculture system was reorganized and Tilapia are not ready to harvest yet.

Alejandro MacDonal prepared a financial analysis assuming a 1500 kg tilapia and 100 kg habanero production (average production of a cycle) having variables such as initial investment, sales, expenses and costs, profits, earnings and finally profitability and investment recovery period. He also conducted a survey of the families involved to determine how the system would integrate to the entire household budget (time and financial).

UJAT demonstration integrated system

Pickled habeneros

We trained the group of Caridad Guerrero about producing a value added product with the habanero production. Specifically, we helped with a pickling process that preserves and increases the value of the habaneros from 40 pesos per kg to 200 pesos per kg. The peppers are put into bottles with oil, vinegar, local herbs and spices.

To provide an enterprise model documenting the benefits of the integrated system
A social economic evaluation of the community was performed at Caridad Guerrero, based on qualitative statistics with surveys and interviews. Final results are being tabulated and will then be translated to English.

Evaluation of Different Tilapia Strains used in Southeastern Mexico and Incorporation of a Pure GIFT Line as Reference to Determine Quality of Tilapia Fingerlings.
EXPERIMENT 1. Comparison of seven Tilapia lines used in South east Mexico
Five thousand fingerlings were purchased anonymously. Seven strains of Nile Tilapia (*Oreochromis niloticus*) were obtained including: "GIFT", "YY super males", "Chitralada", "Rocky Mountain", "Stirling", " Pucté" and Tabasco line, from different hatcheries and/or retailers. Tilapia sizes were from 0.3 to 1.3 g. Initial weight and total length among the lines was used as co-variables in order to avoid statistical bias. Monthly samples were made in order to evaluate growth in weight and length. 1000 Tilapias were placed randomly in mosquito-mesh hapas for a month, at the end of this period all fish were counted in order to evaluate survival and the tilapias were transferred to ½" mesh hapas. Tilapias were fed three times per day with a ratio of 5% total biomass. Feed ratio was adjusted each month. Water exchange was done at 10% ratio weekly. 2000 Tilapias were used to evaluate possible infections for most common bacterial pathogens and parasites (*Streptococcus, Trichodina, Columnaris,* or *Aeromonas*), ich disease (*Ichthyophthirius multifilis*), and parasites. Five fish were sampled for bacterial infection sampling skin, liver, spleen and kidney. Also five fish were analyzed for parasites diseases analyzing superficial tissue from body and gills. Samples were taken and analyzed by the personnel of the Aquatic Sanitation Laboratory (UJAT).

Statistical analysis
The experimental design contemplated for this experiment was a random block design. Three factors were considered to be blocked (length, weight and date of initiation). The response variables (Length and Weight) were tested to determine if the assumptions for parametric analysis were met; if so, contrasts will be performed using ANOVA, otherwise data will be transformed to meet the requirements. Total biomass

will be compared using an ANOVA test and Survival results will be compared among treatments by Chi square test using contingency tables.

EXPERIMENT 2. Evaluation of three Tilapia lines at commercial farming level
The three best Tilapia lines evaluated in the first experiment were tested at the farm El Pucte del Usumacinta S.A. de C.V., located in the Emiliano Zapata municipality, Tabasco. The fry size was dependent on the size of the system to be used, an estimate of 20,000 fish per line were placed in earthen ponds of 50 x 20 m with two replicates per line with a density of 10,000 fish per pond. The fish were kept in these ponds for six months for growth. Monthly samples were collected of each replicate for six months. Total length and weight were taken of a subsample of 100 fish of each replicate. Fish were fed three times per day at a ratio of 5% total biomass. The amount of feed was adjusted monthly. Water exchange was performed at 10% once a week.

Statistical analysis
The primary variables (weight and length) were tested in order to determine if data match parametric analysis assumptions. In case of matching an ANOVA was performed, in contrary the Kruskall-Wallis test will be performed. The total biomass will be compared using an ANOVA and survival results will be analyzed through Chi-square using contingency tables.

RESULTS

Demonstration and Evaluation of an Integrated Aquaculture – Agriculture System for Indigenous Farmers in Tabasco, Mexico

A total of 80 participants participated in the Integrated aquaculture agriculture and bioflocs workshops. The participants included; professors, students, local farmers, and extension government agents.

In Caridad Guerrero habanero harvest started with early producing plants, the rest are flowering and all still growing, Tilapia reached a medium of 45 g. Nitrogen analysis showed high retention from soil matrix and plants. Oxiacaque system is showing excellent progress, habanero plants reached 18 cm and Tilapia 34 g. At UJAT Tilapia achieved 20g and habanero plants 16 cm. Nitrogen analyses were carried out at Caridad Guerrero and Oxiacaque, where high amounts of nitrogen (around 70%) was retained by soil matrix and uptake by plants. Due to problems with soil compaction, some of the slope was lost and capture of effluents was not precise at the demonstrative site.

It turned out that the coconut husk material was especially useful for trapping the effluent and slowly releasing the liquid and nutrients to the sand/silt/husk mix. The peppers, and other vegetables, quickly grew and the root mass helped to bind the materials and further enhance the effluent capture and nutrient availability. The mix eventually formed a type of soil much faster than were seen in the other vegetable plots that had been scoured in the flood. Nitrogen analysis showed high retention from the soil matrix and plants.

Two surveys and one interview were carried out to 80% (100 families) of the population in Caridad Guerrero in order to achieve data for the social economic analysis and describe the impact of the project at the community.

Evaluation of the success of local farmers adopting multi-use concepts to grow fish and plant crops. The value added product developed in the Caridad Guerrero project (Fig. 8) together with the commercialization of the raw habanero and the Tilapia production are the final products of the system.

The results of the financial analysis showed that the investment recovery period will be in the second year of production, there is a profitability of 0.48 and a average earnings of $23, 308.33 (Fig. 9)

Evaluation of Different Tilapia Strains used in Southeastern Mexico and Incorporation of a Pure GIFT Line as Reference to Determine Quality of Tilapia Fingerlings.
All seven lines were bought that are available in the region: “GIFT”, “super males YY”, “Chitralada”, “Rocky Mountain”, “Stirling”, “Pucté” and “Tabasco line”. Tilapias (1000 per hapa) were placed in mosquito mesh for a month. At the beginning Tilapia were sampled in order to determine differences in length and weight among the lines. At the end of the first month a sampling was conducted in order to evaluate growth. Fish were counted and placed in a ½” mesh hapas.

In the present study the statistical analysis indicates the existence of statistic differences among the different Tilapia Lines at the beginning of the trial (ANOVA; $p<0.01$; Fig. 1). The line three showed initially the best results in weight with 1.38 ± 0.01 g and the line with the lowest weight was the four with 0.28 ± 0.01 g. Length showed the same pattern (ANOVA; $p<0.01$; Fig. 2) with 4.28 ± 0.01 cm for line three and 2.41± 0.01 cm for line four.

The multifactorial analysis indicated that there is no statistical difference among Tilapia lines (ANCOVA; $p < 0.87$) for the first sampling month, being the line three the highest on weight with 1.68 ± 0.97 g, while line four showed the lowest value with 0. 31 ± 0.34 g. However, the covariable initial weight of the experiment did not have any effect on the organisms weight (ANCOVA; $p = 0.87$; Fig. 3). In the case of length, results showed that there is statistical difference among lines tested in this study (ANCOVA; $p < 0.04$ Fig. 4) due to best initial length was on fish of line three with 9.19 ± 0.36 cm and the lowest the line four with 6.57 ± 0.14 cm. The covariable initial length did not have an effect over length (ANCOVA; $p = 0.77$).

In the second month of sampling the results showed that there is no statistical differences among the Tilapia lines (ANCOVA; $p < 0.35$; Fig. 5), line three showed the best growth in weight with 27.80 ± 2.98 g and line four showed the lowest weight with 15.26 ± 5.91 g. This statistical analysis allow to identify that initial weight did not affect growth in weight among the different Tilapia lines tested (ANCOVA; $p = 0.32$). The analysis for length indicated that there is an effect highly significative among Tilapia lines of this study (ANCOVA; $p<0.01$; Fig. 6). The line three showed the best results with 11.48 ± 0.60 cm length and line four the lowest results with 9.02 ± 1.05 cm. The covariable initial length did not affect the results of growth (ANCOVA; $p = 0.13$).

For the third month of sampling the statistical analysis showed significant differences (ANCOVA; $p = 0.04$; Fig. 14) the Tilapia line one showed the best growth in weight with 82.79 ± 6.26 g. The line with the lowest growth weight was the line six with 52.08 ± 8.30 g. The analysis also indicated that there was no effect of the initial weight with respect to fish growth (ANCOVA; $p=0.22$). For length the analysis indicated that there are significant differences (ANCOVA; $p=0.05$; Fig. 15) between the different Tilapia lines, the best line with length growth was the line three with 16.71 ± 1.63 mm and the lowest length for line six with 13.24 ± 1.23 mm. The covariable initial length did not had an effect on fish growth (ANCOVA; $p=0.22$).

For the fourth month of sampling the statistical analysis indicated that there is statistical differences between the Tilapia lines (ANCOVA, $p<0.01$; Fig. 16 and 17) for weight and length, the line one (118.40 ± 14.35 g) showed the highest weight and the line seven showed the lowest growth (64.93 ± 6.32 g). For length the results are similar with 18.24 ± 0.70 cm for line one having the best results and 13.83 ± 0.29 cm for line four showing the lowest length. There was no effect of density over growth (ANCOVA, $p= 0.09$). For the fifth month of sampling the analysis indicated statistical differences between the Tilapia lines (ANCOVA, $p<0.01$; Fig. 18 y 19) for weight and length. There was no effect of density over growth (ANCOVA, $p= 0.42$) for weight and length. The line one had the best growth (224.41 ± 18.37 g) and the lowest line was the four (91.47 ± 15.46 g) the line one had the best length with 24.88 ± 1.06 cm and the lowest was the line four with 12.57 ± 0.69 cm.

The average value of temperature, pH and dissolved oxygen were 29.9 ± 4.52 °C, 7.22 ± 0.31 y 3.86 ± 4.41, respectively.

The results of the bacteriology tests showed the constant presence of two types of bacteria *Pseudomonas fluorescens* y *Aeromonas hydrophila* in most of the Tilapia lines tested on this experiment (Table 1).

A Chi-square with contingency tables analysis was carried out in order to determine differences in percentage of masculinization rates of the seven Tilapia lines tested in the study. The analysis showed that there is statistical differences among Tilapia lines for percentage of masculinization (X^2; $p <0.0$), being line six the best result in masculinization with 98%, while line with the lowest percantge of masculinization was line five with 82% (Fig. 7).

DISCUSSION

The results in the present study indicated that the growth of the seven Tilapia lines evaluated and which initiated with differences in weight and size was not significative, by contrary Orozco 1998 and Castro et al., 2004 found statistical differences using Tilapia frys of weight under 0.5 g when comparing three different lines of Tilapia.

In which respect to the growth in weight of the seven Tilapia lines there was no significative differences at the thirty first days of the experiment between the different lines, the Tabasco line had the best growth in weight and size. For the second month of sampling (60 days) the obtained results were very similar for growth, the Tabasco line had the best growth in weight and size, Castro et al., 2004 found statistical differences during the first and second month of experiment in growth of the three Tilapia lines evaluated being for this case *Oreochromis mossambicus* the best in growth in weight and size.

Orozco 1998 described that smaller sizes could be associated with mortalities caused by predators and parasites, this could explain the mortalities we had in our study where Tilapia mortalities were associated to parasites and bacterial infection during the first month of the experiment.

We plan to publish these results in a peer reviewed science article once the trial is completed in the Oxiacaque village and supported with additional data collection of soil quality and nutrient levels. We were asked to present our findings at an aquaponics conference in Cancun, Mexico. Fitzsimmons made one presentation on September 22, 2011 and two graduate students from Universidad Juarez Autonoma de Tabasco made a separate presentation on September 23, 2011. The presentations specifically mention the support of USAID and the AquaFish Collaborative Research Support Program.

Mercado Mundial en la Producción de Tilapia (World Market Tendencies of Tilapia Production). – Dr. Kevin Fitzsimmons, USA

and

DESARROLLO DE PRACTICAS DE ACUACULTURA SUSTENTABLE EN TABASCO, MÉXICO USANDO TECNOLOGÍA DE SISTEMAS INTEGRADOS AGRÍCOLAS – ACUÍCOLAS (Developing Sustainable Practices for Aquaculture in Tabasco Mexico, Using Integrated Agriculture-Aquaculture Integrated Systems Technology). – María Fernanda Cifuentes Arroyo and Maria Contreras.

In January 2012, Fitzsimmons returned to Tabasco to attend Rafael Martinez's wedding and was interviewed by local newspaper regarding the project. See appendix.

http://www.diarioavancetabasco.com/Default.aspx?ClaveIdioma=1&Noticia=6&Clave=1&Fecha=10/01/2012

Aquaponics bed with habanero peppers

Tilapia at first sampling date

LITERATURE CITED

Chan, G.L. 1993. Aquaculture, ecological engineering: lessons from China. Ambio 22:491-494.

Rakocy, J., Hargreaves, J. and Bailey, D. 1993. Nutrient accumulation in a recirculating aquaculture system integrated with hydroponic vegetable production. pp. 148-158 In: Techniques for Modern Aquaculture (J.K. Wang, Ed.) St. Joseph, MI:ASAE Pub. 02-93.

Singh, S., Marsh, L.S., Vaughan, D.H., Libey, G.S., 1996. A computer simulation model to optimize greenhouse size for an integrated (fish production, hydroponics) system. Transactions of the ASAE 39, 2241–2248.

U.S. Environmental Protection Agency (USEPA). 2002. Development Document for Proposed Effluent Limitations Guidelines and Standards for the Concentrated Aquatic Animal Production Industry Point Source Category. U.S. Environmental Protection Agency, Washington, DC.

Williams, M.J., 1997. Aquaculture and sustainable food security in the developing world. In: Bardach, J.E. (Ed.), Sustainable Aquaculture. Wiley, New York, pp. 15–51.

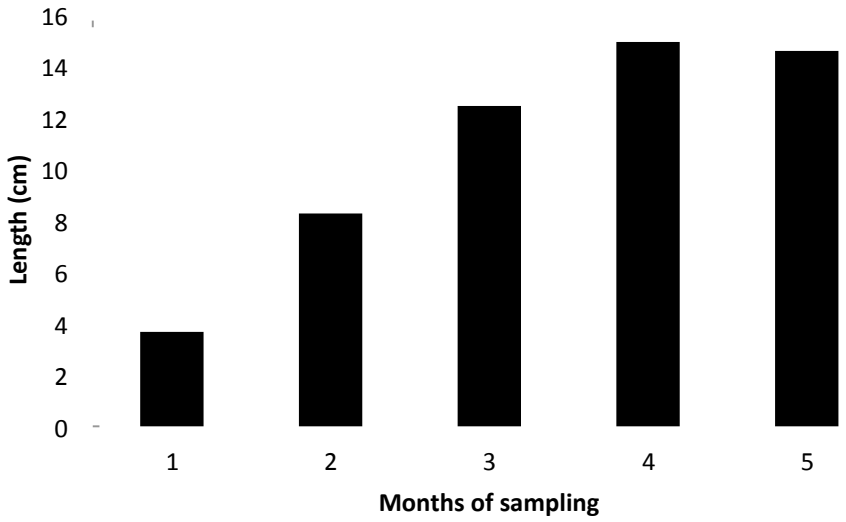


Figure 1. Total growth length of Tilapia from Caridad Guerrero system

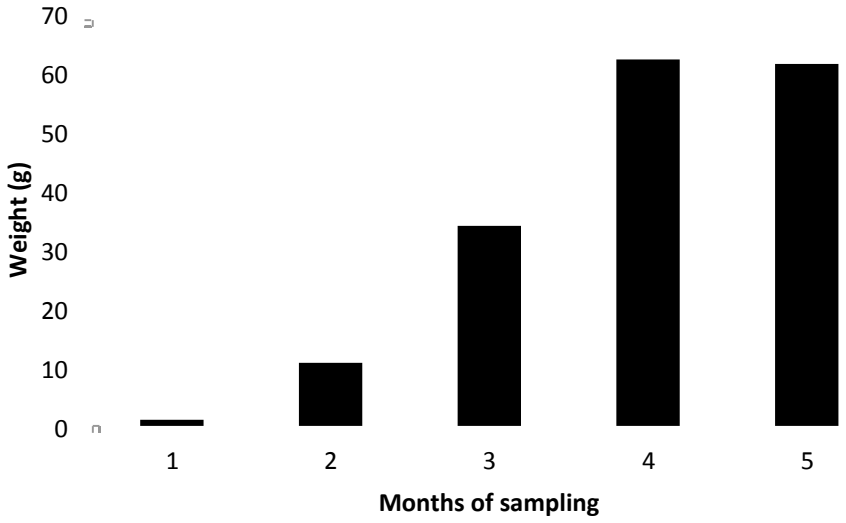


Figure 2. Total growth weight of Tilapia from Caridad Guerrero system

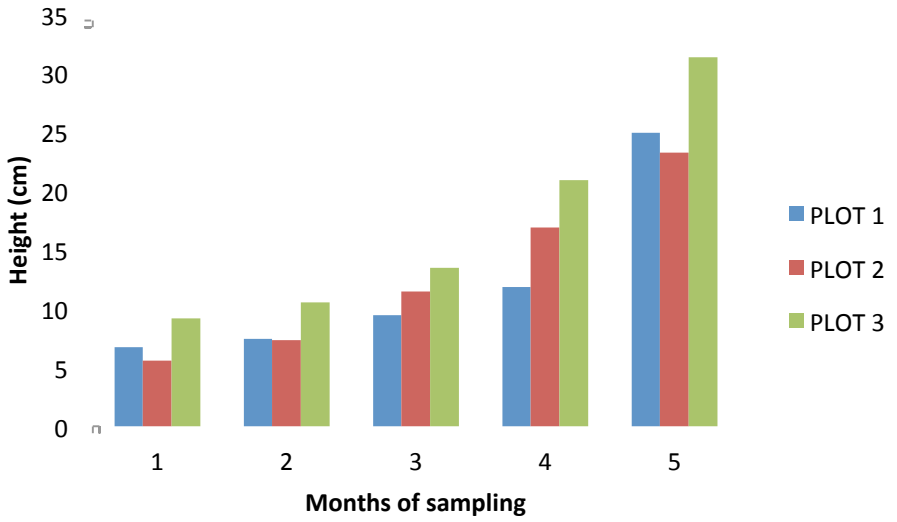


Figure 3. Growth in height of Habanero plants for the five months of sampling at Caridad Guerrero system

	NITRATES mg/L	NITRITES mg/L	AMMONIA mg/L
Tilapia tank	0.0	0.16	0.45
PLOT 1	4.0	0.13	0.74
PLOT 2	3.5	0.10	0.74
PLOT 3	4.0	0.13	0.46

Figure 4. Water quality parameters from different source at Caridad Guerrero system for August

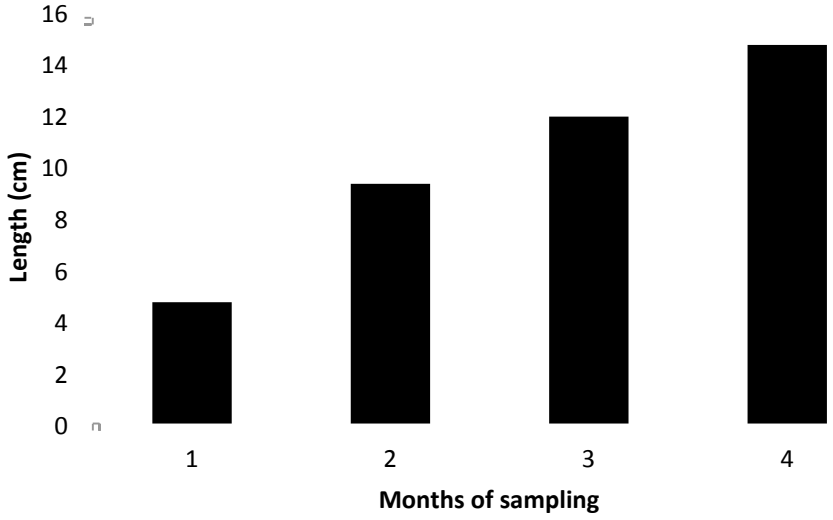


Figure 5. Total growth length of Tilapia from Oxiacaque system

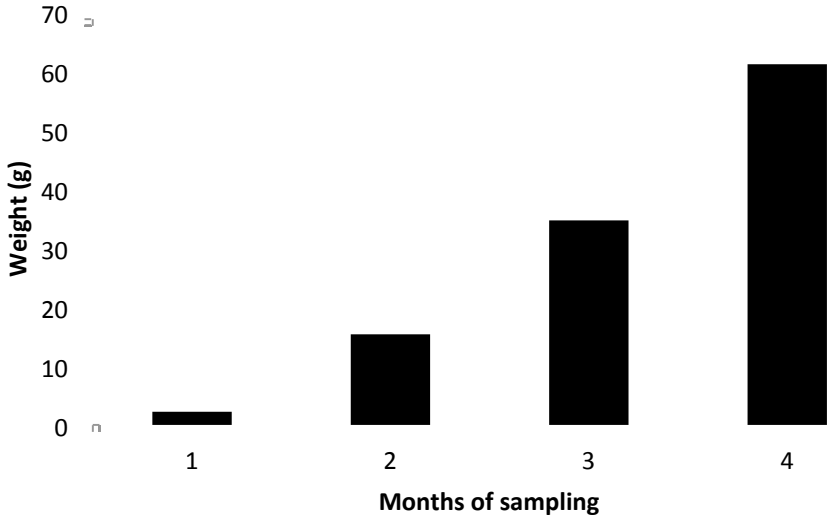


Figure 6. Total growth weight of Tilapia from Oxiacaque system

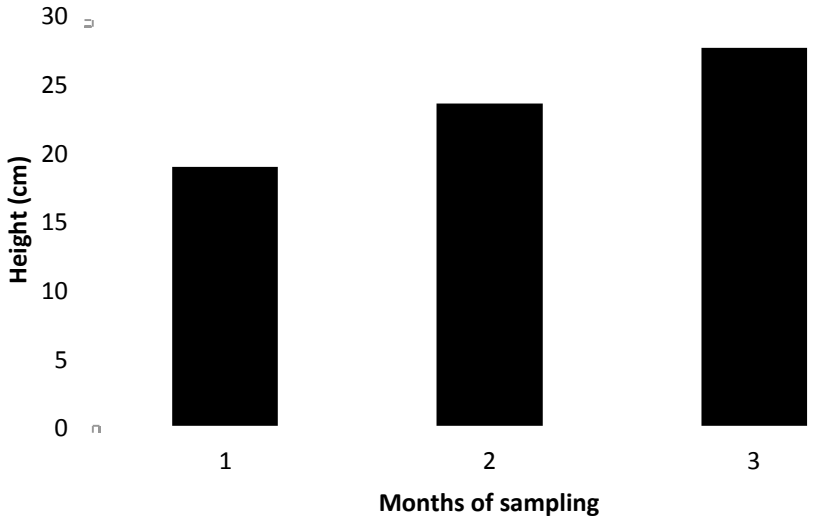


Figure 7. Growth in height of Habanero plants for the three months of sampling at Oxiacaque system

Figure 8. Pickled Habanero developed by farmers of Caridad Guerrero group

Concept	0	1	2	3	4	5
Initial investment	**$47,925**					
Sales	$38,220	$38,220	$64,470	$64,470	$64,470	$64,470
Expenses and costs	$47,925	$25,525	$30,250	$30,250	$30,250	$30,250
Profits	$-9,705	$12,695	$34,220	$34,220	$34,220	$34,220
Earnings	$-9,705	$2,990	$37,210	$71,430	$105,650	$139,850

Average earnings	$23,308
Profitability	0.48
Investment Recovery Period (years)	2.05

Figure 9. Financial Analysis of the aquaponia production for the Caridad Guerrero system

Table 1. Bacterial and parasitic diseases presents in the seven Tilapia lines of the experiment

Line	Bacteria found	Parasites found
1	*Pseudomonas fluorescens, Aeromonas hydrophila.*	Monogene and tricodina
2	*Pseudomonas fluorescens, Aeromonas sobria, Aeromonas hydrophila.*	Monogene and tricodina
3	*Pseudomonas fluorescens, Plesiomonas shigelloides, Aeromonas sobria, Moraxella spp y Aeromonas hydrophila.*	Monogene and tricodina
4	*Aeromonas hydrophila, Aeromonas sobria,Plesiomonas shigelloides y Pseudomonas fluorescens.*	Absent
5	*Aeromonas sobria, Pseudomonas putida, Plesiomonas shigelloides, Pseudomonas aeruginosa y Pseudomonas fluorescens.*	Monogene
6	*Pseudomonas fluorescens, Aeromonas hydrophila, Burkholderia cepacia, Pseudomonas putida y Photobacterium damselae.*	Monogene and tricodina
7	*Pseudomonas putida, Aeromonas hydrophila y Pseudomonas aeruginosa.*	Monogene, tricodina and other protozoa, unidentified parasites ciliates

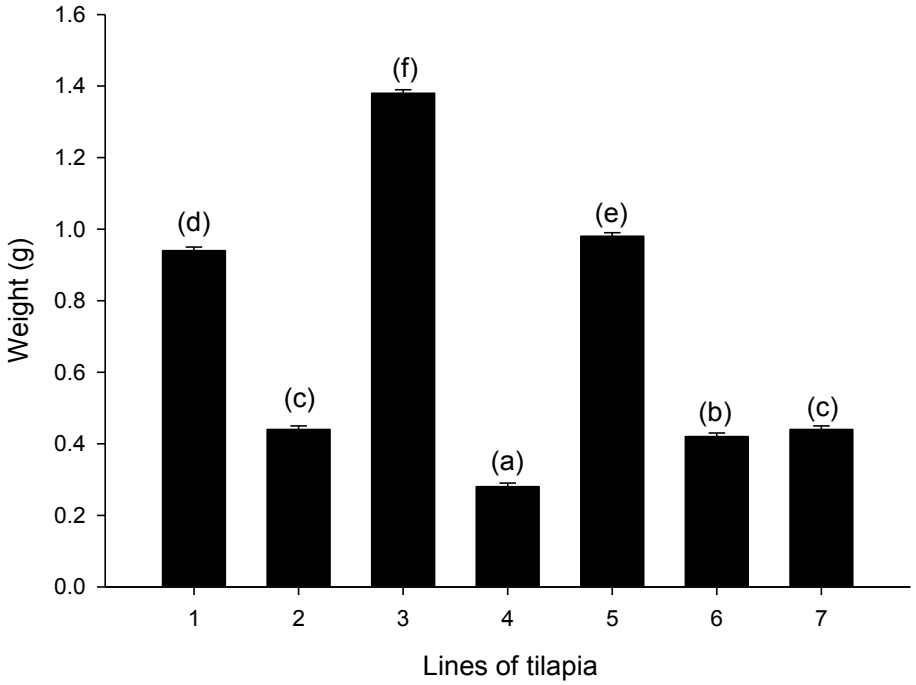


Figure 8. Initial weight of seven Tilapia lines tested in this study

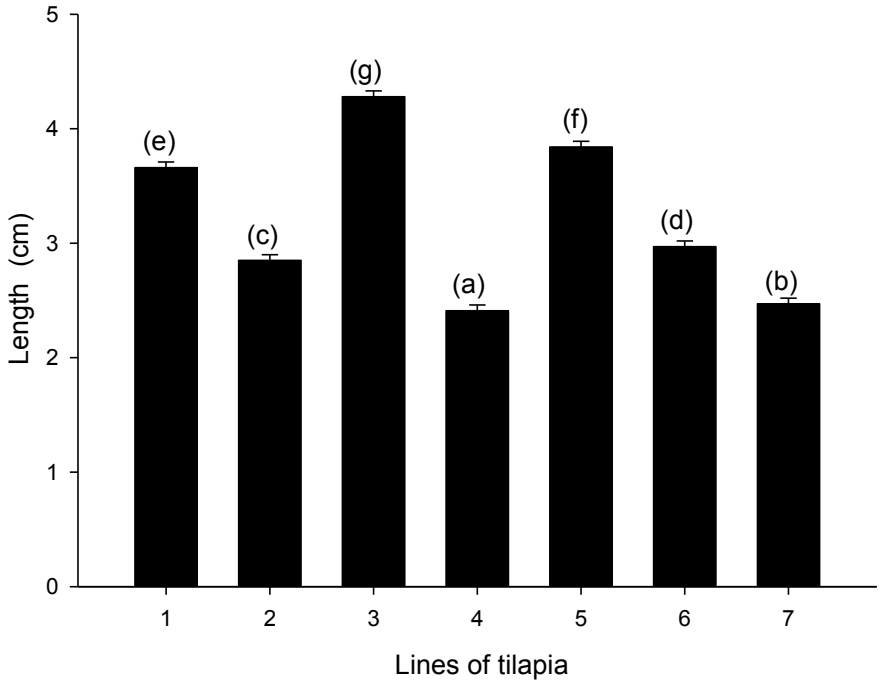


Figure 9. Initial total length of the seven Tilapia lines tested on this study

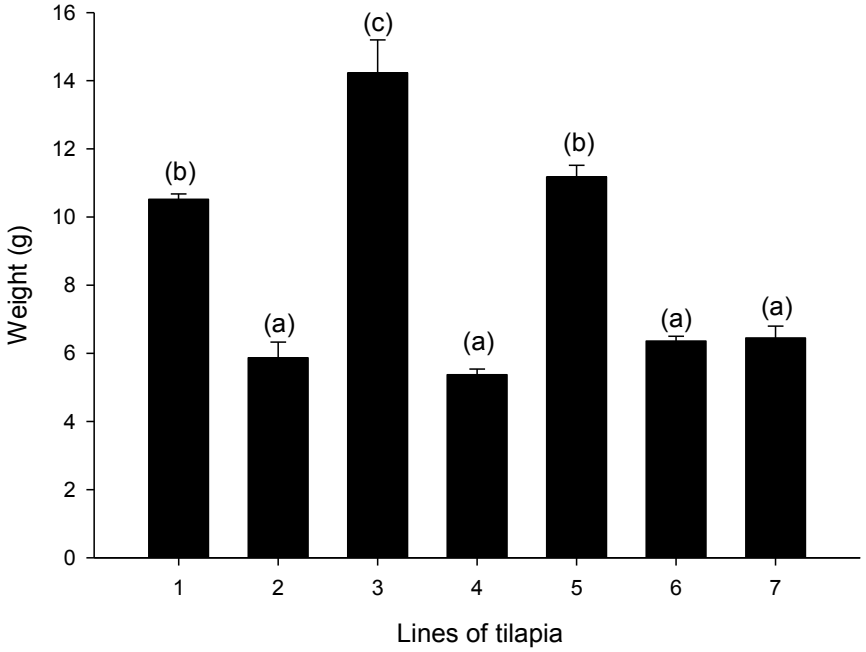


Figure 10. Weight growth of the seven Tilapia lines evaluated in this study during the first month of the experiment

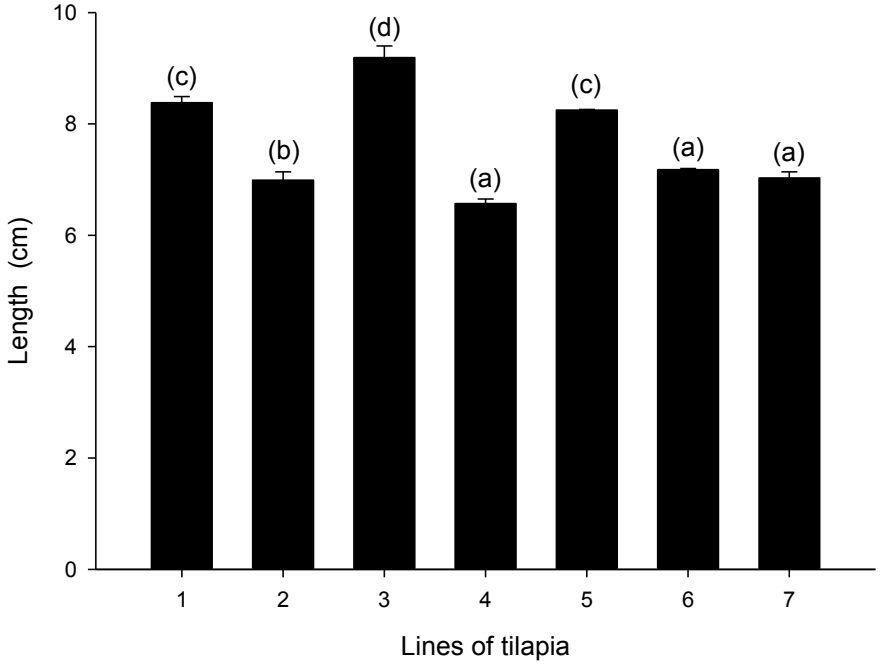


Figure 11. Growth in length of the seven Tilapia lines tested in this study during the second month of the experiment

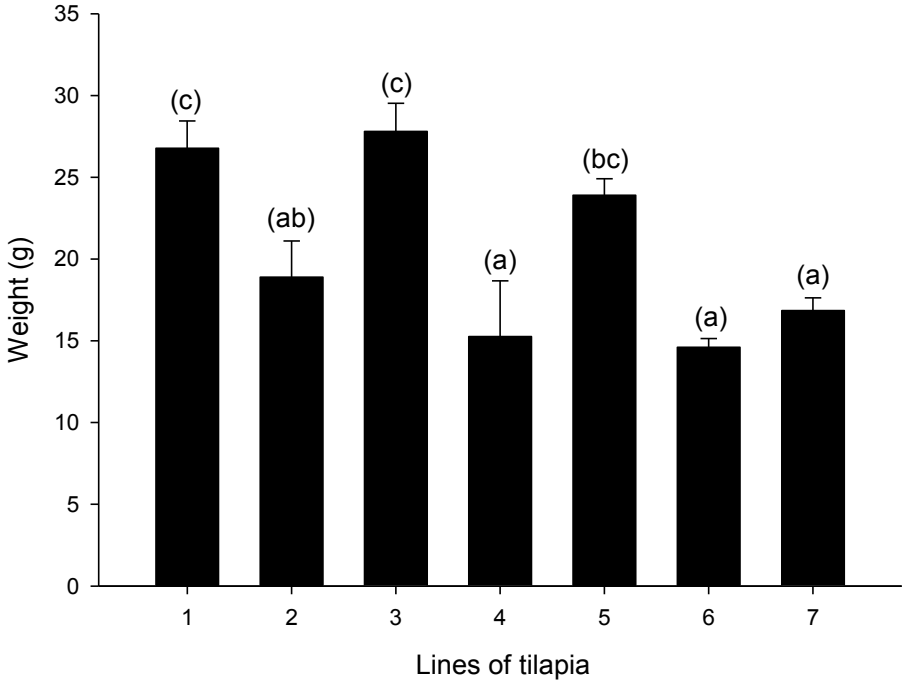


Figure 12. Growth in weight of the seven Tilapia lines tested during the second month of this experiment

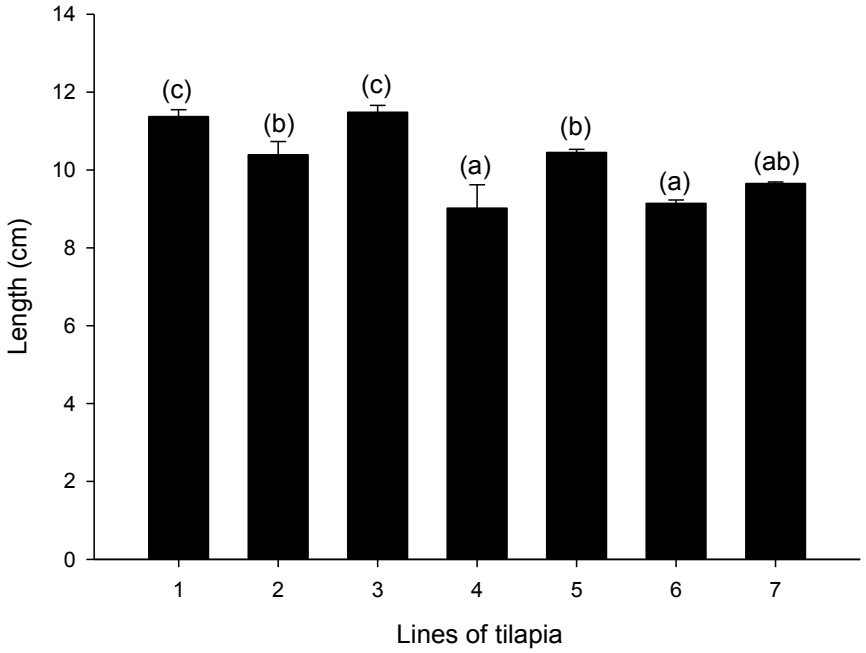


Figure 13. Growth in length of the seven Tilapia lines tested in this study during the second month of the experiment

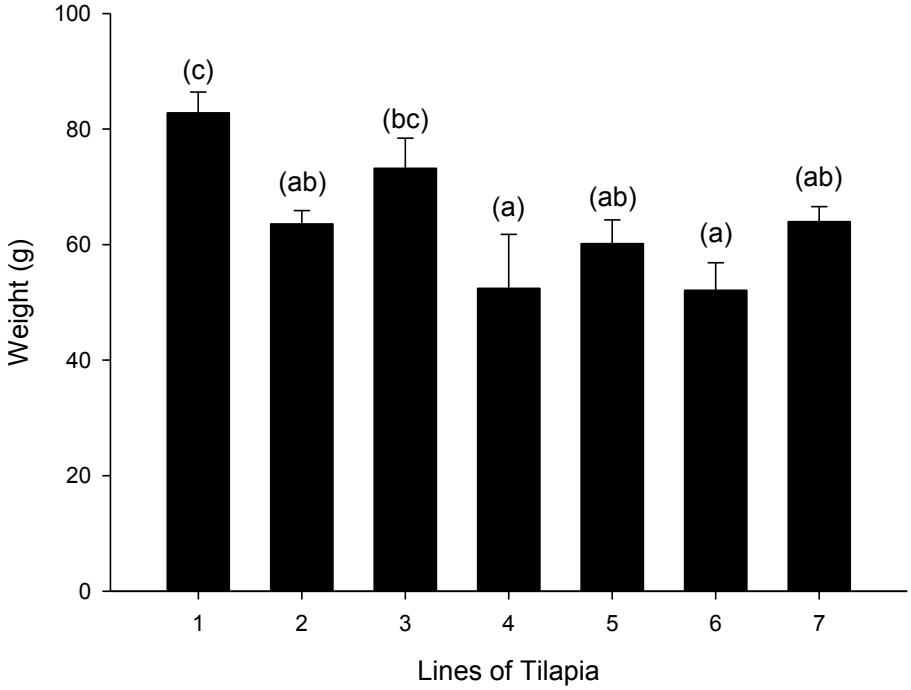


Figure 14. Growth in weight of the seven Tilapia lines tested in this study for the third month

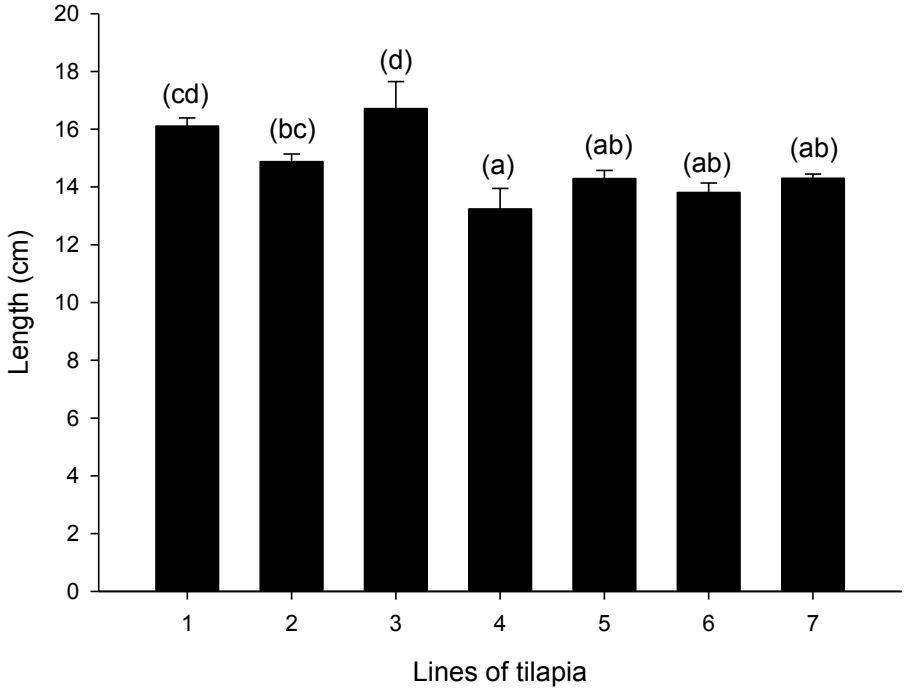


Figure 15. Growth in length of the seven Tilapia lines tested in this study for the third month

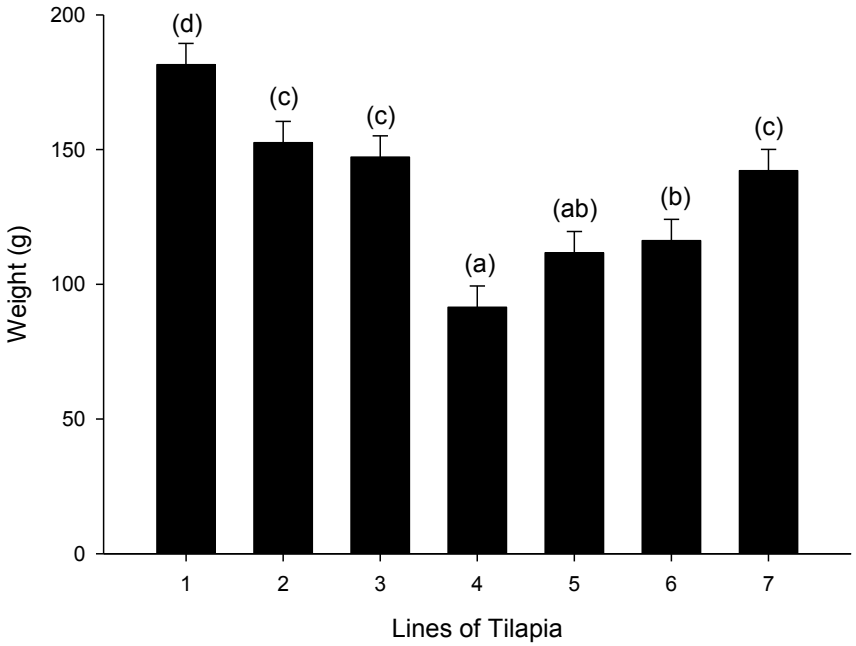


Figure 16. Growth in weight during the 120 days of the experiment for the seven Tilapia lines tested in this study.

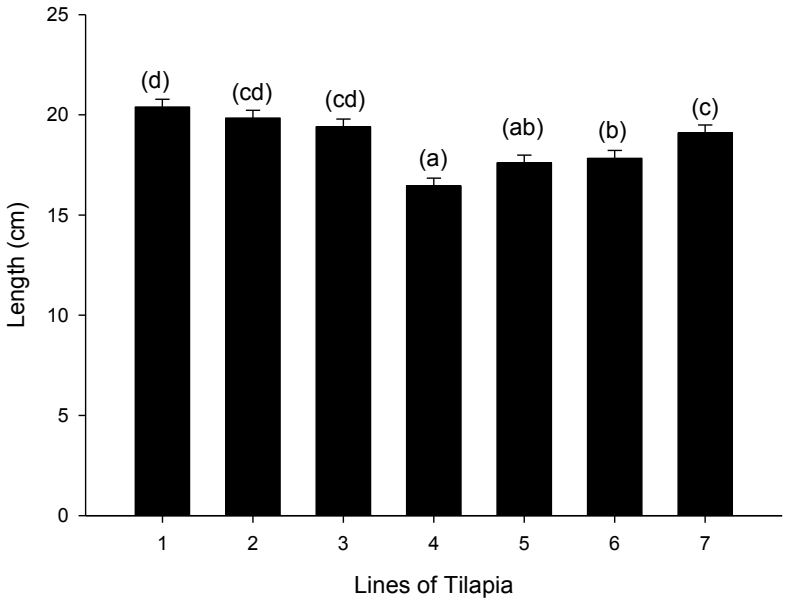


Figure 17. Growth in length during the 120 days of the experiment for the seven Tilapia lines tested in this study.

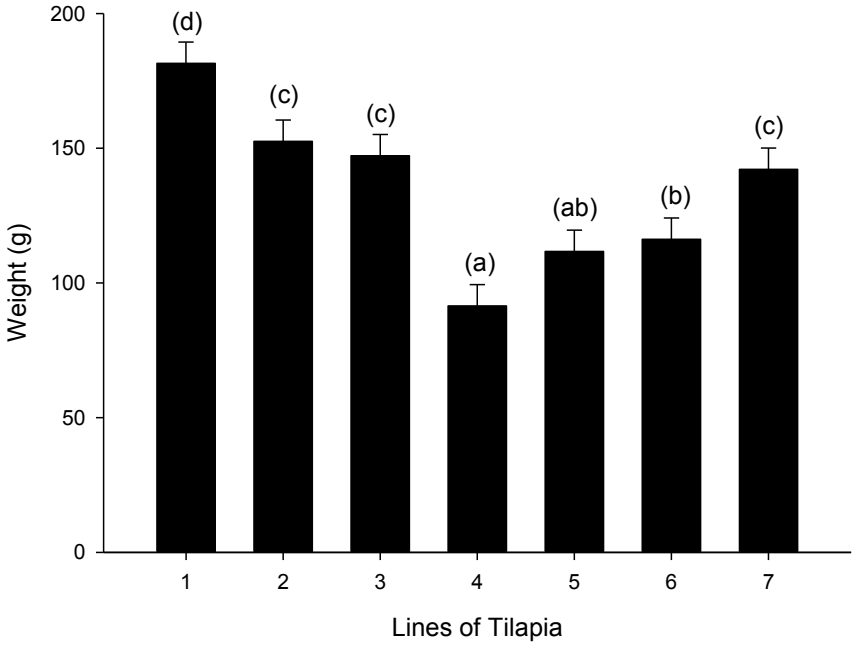


Figure 18. Average growth value in weight of the seven lines of Tilapia during the 150 days of the experiment

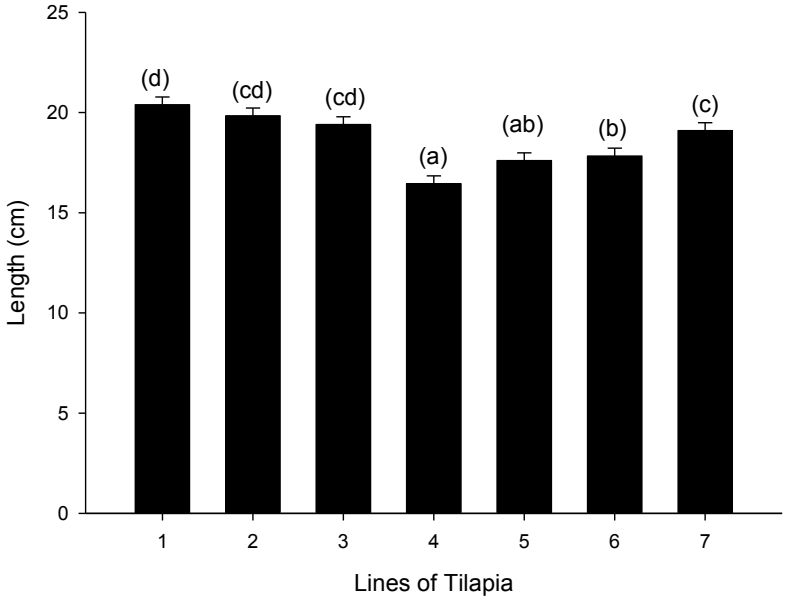


Figure 19. Average growth value in length of the seven lines of Tilapia during the 150 days of the experiment

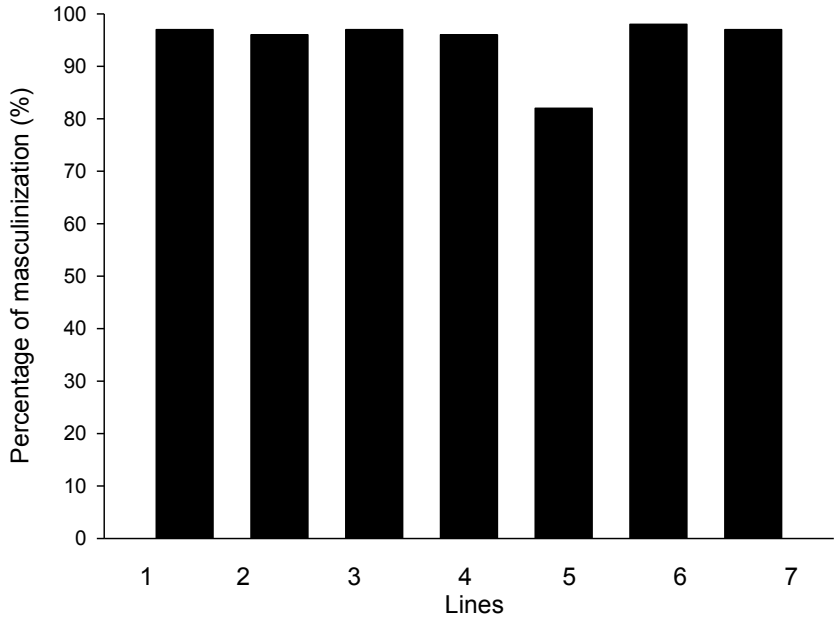


Figure 20. Percentage of masculinization of the seven Tilapia lines tested in this study (n=100 for each Tilapia line)

APPENDIX 1

Universidad Juárez Autónoma de Tabasco
División Académica de Ciencias Biológicas
Laboratorio de Acuicultura Tropical
Workshop on Integrated Aquaculture Agriculture Systems

Agenda

Registration 9-10 AM

AUGUST 12, 2010		
Time	**Topic**	**Instructor**
10:00-12:30	Introduction Concepts of Integrated Aquaculture Agriculture Systems (IASS) Practical activity	PhD. Candidate Rafael Martínez García
12:30-13:00	**Break**	
13:00-14:00	Historical perspective IAA projects on development	
AUGUST 13		Dr. Dennis McIntosh/ Dr. Kevin Fitzsimmons
9:00- 12:00	Principal objectives of the IAAS Function and development of IAAS Implementation of IAAS	
12:00-13:00	**Break**	
13:00-15:00	Actual status of IAAS projects around the World * Special participation Dr. Kevin	
August 14		
9:00- 14:00	field trip	
August 16		Dr. Dennis McIntosh
9:00-11:00	Organic cycles involved in IAAS	
11:00-12:00	IAAS Project engineering	
12:00-13:00	**Break**	
13:00-14:00	Presentation of practical activity	
August 17		Dr. Dennis McIntosh
9:00-12:00	Development process of IAAS	
12:00-13:00	**Break**	
13:00-14:00	Future perspectives of IAAS	
14:00	Closing	

Diario Avance Tabasco 10 January 2012 By: Luis Garcia de la Cruz

LA UJAT POR UNA COMBINACIÓN DE ACUACULTURA Y AGRICULTURA
Explica Kevin Fitzsimmons, investigador de la Universidad de Arizona los proyectos que existen con la División de Biología.

Kevin Fitzsimmons, investigador de la Universidad de Arizona.

FOTO: Avance

LUIS GARCÍA
SEGUNDA Y ÚLTIMA PARTE

Luego de reconocer que México tiene mucho interés en la exportación de productos del mar a través de la acuacultura, prevalece siempre la necesidad de soportar el consumo local, externó el investigador de la Universidad de Arizona, Kevin Fitzsimmons quien destaca el trabajo que mantiene la Universidad Juárez Autónoma de Tabasco en ese sentido.

Cuestionado sobre el papel que juega la máxima casa de estudios el investigador en acuacultura, indicó que el trabajo que se realiza con la Universidad Juárez y en especial con la División de Biología, donde existen proyectos en coordinación con el doctor Wilfrido Contreras, son de suma importancia por todo lo avanzado, porque se desarrolla de manera rápida, la domesticación de peces, camarones, ostiones, almejas y las algas.

“Algo que la UJAT y el doctor Wilfrido (Contreras), han estado trabajando en acuacultura y agricultura. La acuacultura tiene similares problemas a la agricultura, pues también causa contaminación, necesitamos encontrar alimentos para los peces, para ir eliminando las harinas de pescado por ser muy caras y no es suficiente para atender la alimentación de las especies domesticadas”.

Por ello, agregó, decidimos poner la acuacultura y la agricultura juntos, es mejor para la "Revolución azul", que es la conjugación de la acuacultura y la agricultura, explica ante la presencia de sus colegas tabasqueños que escucharon atentos la explicación del especialista de la Universidad de Arizona.

Y refrenda que es usar el agua que no sirve para los peces, utilizarla para irrigar las plantas, pues los desechos de los peces son muy orgánicos, pues tienen potasio, que las plantas necesitan y el productor puede ahorrar dinero, no se contamina el ambiente, porque son desechos de peces y no se usan fertilizantes. Un sistema similar que se usa con camarones y con algas marinas.

Sostiene que es un sistema nuevo en América, tiene su prototipo en China, Japón, que han tenido por largos años la siembra de arroz y pescado, "ellos tienen el uso de plantas y peces juntos".

Indica que los investigadores norteamericanos y mexicanos explican a los granjeros como atender la tierra, pero en China, la gente no entiende los ciclos de los nutrientes, nosotros hemos trabajado con las comunidades, nuestros estudiantes se involucran con las comunidades para mostrar nuestros sistemas, dan asesorías, demostraciones, talleres en el campus.

Precisa que en Tabasco se trabaja en Tacotalpa, en la comunidad de Caridad Guerrero; en Nacajuca en Oxiacaque, y un sistema en el campus universitario. Mientras que en otros estados, se trabaja con Chiapas, Oaxaca, Tamaulipas, Veracruz. Se trabaja con tilapias aunque el concepto es para todas las especies, también en Jalapita, Centla, se investiga con algas, camarón, manglar, ostión.

Refirió que en el proyecto participan todos los profesores, todos los alumnos, todos los administrativos de acuacultura, es algo único en México. Porque está enfocado hacia la triple hélice, empresa, gobierno y universidad. Es importante el proyecto porque se logró traer a otros investigadores e instituciones. "Unas de las cosas por las que me gusta trabajar con los colegas de México, de aquí de la Universidad, es porque México le ha apostado más fuerte a la acuacultura que el mismo Estados Unidos, la industria de la tilapia ha crecido muy rápido, camarón, bagre en el norte, un programa muy fuerte para trabajar con las especies nativas como robalo, tenguayaca, casta rica, paleta y el pejelagarto, por supuesto".

Cuestionado sobre las perspectivas de desarrollo en materia de educación de la acuacultura y sobre todo de México, dijo que a través de programas como los que se están tratando de reproducir aquí en Ciencias Biológicas y explica que en Estados Unidos, se han comenzado a trabajar con las preparatorias, colocando estanques, peceras para que los muchachos aprendan a cuidar a los peces, principalmente en Arizona, porque la mayor parte de las secundarias y prepas están en áreas rurales. Antes, estudiaban vacas, cerdos, pollos, pero las ciudades han crecido y ahora las escuelas ya están dentro del perímetro de la ciudad en zonas urbanas. Con la acuacultura se pueden enseñar la crianza, mantenimiento y gestión de granjas y se pueden usar en sus clases de cocina. Pero lo más importante es que las personas dedicadas a la protección de animales, no se quejan porque son criaderos.

Explicó que uno de los egresados del programa está trabajando en una escuela y ha comenzado a desarrollar esa misión. "Bueno, realmente no es una acción integrada, pero espera que con esa visión que tiene del programa más adelante se pueda reproducir esa forma de trabajo. Es el comienzo. Una de las cosas que admira de la UJAT es que todos los participantes, lo tienen muy claro, que tienen que tener una solida formación", concluye Kevin Fitzsimmons.

Development of Polyculture Technology for Giant Freshwater Prawns (*Macrobrachium rosenbergii*) and Mola (*Amblypharyngodon mola*)

Quality Seedstock Development/Experiment/09QSD03UM

Md. Abdul Wahab
Bangladesh Agricultural University
Mymensingh, Bangladesh

Liu Liping
Shanghai Ocean University
Shanghai, China

James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

ABSTRACT

This project evaluated potential developments in prawn polyculture by testing a variety of polyculture systems for growth and yield. Experiment 1 tested the effects of selective harvesting (SH) and claw ablation (CA) of blue-clawed (BC) prawns on an all-male freshwater prawn-finfish polyculture system. Ponds were stocked with all-male freshwater prawn *Macrobrachium rosenbergii*, silver carp *Hypophthalmichthys molitrix*, catla *Catla catla* and mola *Amblypharyngodon mola* at 12000, 2000, 500 and 20000 per ha, respectively. Prawns were fed with pelleted feed. Ponds were fertilized regularly with urea, triple super phosphate and cow-dung. SH of BC prawns in treatment SH and CA in treatment CA started on the 60th day during a 137-day culture and continued at 15-day intervals until the final harvest. Treatment SH resulted in a higher ($P<0.05$) net production of freshwater prawn (437 kg/ha), with better survival and mean weight, followed by CA (354 kg/ha) and Co (322 kg/ha). The combined net production of prawn plus finfish was also higher in SH (1244 kg/ha) as compared with CA (1161 kg/ha) and Co (1137 kg/ha), although the finfish production did not differ significantly.

In experiment 2, the potential of addition of the nutrient-dense, small fish mola into polyculture with freshwater giant prawn was evaluated at the Fisheries Field Laboratory, Bangladesh Agricultural University, Mymensingh during August to December 2010. The effects of mola at different densities in polyculture with freshwater prawn, and the production performance between all-male and all-female freshwater prawn in monoculture were evaluated. The experiment had five treatments: all male prawn + 1 mola m^{-2}, all male prawn + 1.5 mola m^{-2}, all male prawn + 2 mola m^{-2}, only male prawn and only female prawn (T_1, T_2, T_3, T_4 and T_5, respectively) with three replications each. Prawn stocking densities were the same (3 juvenile m^{-2}) in all treatments. Feeds were supplied twice daily for prawn in all ponds at appropriate feeding rates. Plankton, macro-benthos abundance and water quality parameters (except transparency and chlorophyll-a) did not vary significantly ($P>0.05$) among the treatments. Survival of prawn in monoculture irrespective of gender was lower than in polyculture with mola. Mean harvest weight, weight gain, specific growth rate, net and gross production of all female prawn was significantly ($P<0.05$) lower than all male and those of other treatments with different densities of mola. Addition of mola at different densities had no effect on survival, gain in weight, or production performance of freshwater prawn. Therefore, mola may be stocked as an additional species with freshwater prawn that would give higher total production, provide family nutrition as well as generate additional income.

In experiment 3, the effects of adding silver carp and catla to mola and freshwater giant prawn in polyculture systems were evaluated. The experiment had five treatments: T_1 (prawn and mola), T_2 (prawn, mola and catla), T_3 (prawn, mola and silver carp), T_4 (prawn, mola, catla, and silver carp), and T_5 (only mola), each with three replications. Stocking density of mola varied to produce approximately the same total fish biomass in all the treatments. Prawn stocking densities were 120 juvenile dm^{-1} (dm=decimal $=40m^2$) and 6 carp dm^{-1}. Feeds were supplied twice daily for prawn at appropriate feeding rates. Water quality parameters (except transparency) did not vary significantly among treatments. Survival of freshwater prawn in prawn-mola-carps polyculture system were relatively higher where prawn and mola were stocked with silver carp and catla, and with silver carp, respectively than with only mola or with mola and catla. Net and gross production of prawn were significantly higher with silver carp and with silver carp-catla but not with only mola or mola-catla. Average weight gains, SGR, net and gross production were significantly lower for silver carp than in catla or in catla-silver carp treatments. The balanced stocking densities of prawn-mola with catla - silver carp developed a synergistic interaction resulting higher net and gross productions. Net and gross productions were significantly higher in treatment T_5, where only mola was stocked at higher densities. Therefore, carps can be added with prawn and mola to enhance total production, which would play a significant role in providing family nutrition as well as generating additional income.

Experiment 1: Effects of Selective Harvesting and Claw Ablation on All-Male Freshwater Prawn Production

INTRODUCTION

The present study was carried out to determine the effects of bi-weekly selective harvesting (SH) and bi-weekly claw ablation (CA) of blue-clawed (BC) freshwater prawns on the growth and production of all-male prawn *Macrobrachium rosenbergii* stocked in polyculture systems with silver carp *Hypophthalmichthys molitrix*, catla *Catla catla* and mola *Amblypharyngodon mola*.

MATERIALS AND METHODS

The experiment was conducted using a completely randomized block design in 12 earthen ponds (nine ponds each with 100m2 and three ponds each with a150 m2 area) having1.0m water depth at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, during 137 days. The experiment included three treatments: SH, CA and SH+CA of all-male freshwater prawn and a control (Co) without SH and CA. Each treatment had four replicas. All ponds were treated with lime (powdered $CaCO_3$) before stocking, and fertilized with urea and triple super phosphate (TSP) at the rate of 25 kg/ ha and semi-decomposed cow-dung at 1000 kg/ha 13 days after liming. Seven days after fertilization, all ponds were stocked with all male prawn juveniles (mean weight 5.58 g each), silver carp (17.93 g), catla (23.07 g) and mola (0.52 g) at stocking densities of 1.2, 0.2, 0.05 and 2.0 per m^2 respectively. Male prawn juveniles were selected manually. Freshwater prawns were fed with pellets (28% crude protein) daily at a rate of 6% of body weight for the first month, 4% for the second month and 3% for the rest of the culture period. Half of the daily ration was supplied in the morning at 8:00 and the rest in the evening at 17:00. Ponds were fertilized at 5-day intervals with urea and TSP at a rate of 6.25 kg/ha and cow-dung at 125 kg/ ha to maintain natural food organisms.

Periodic selective harvest of BC from treatment SH and ablation of BC in treatment CA were started on day 60 after stocking. These procedures were continued every 15 days until the final harvest. BC prawns were identified by their blue-colored and long. A seine (1.0 cm mesh size) was used for SH and CA of BC. Seining was performed twice at each sampling time in the respective ponds. All seined BC in treatment SH were harvested, and blue claws of all seined BC in treatment CA were ablated. Selectively

harvested BC prawns were not replaced, while claw-ablated BC prawns were released immediately into the respective ponds. Neither SH nor CA of BC prawns was performed in the control. Partial harvesting of larger mola using the same seine (1.0 cm mesh size) was started on day 75 and continued at 15-day intervals until the final harvest, because mola bred within 60-70 days of stocking in all ponds. The counts and weights of selectively harvested and claw-ablated prawns as well as the weights of partially harvested mola were recorded.

All prawns, silver carp and catla harvested from each pond were counted, measured and weighed individually. Mola of each pond were batch weighed. Survival rate, specific growth rate (SGR) and individual weight of this species were not considered for calculation as mola were self recruiting and bred in all ponds during the culture period. The specific growth rate and feed conversion rate (FCR) of prawn, silver carp and catla was calculated.

One-way analysis of variance (ANOVA) was performed for the statistical analysis of growth and production. Survival and per cent data were analyzed using arcsine-transformed data but per cent values are reported. All statistical tests were carried out at a 5% significance level using Statistical Package for Social Science (SPSS).

RESULTS AND DISCUSSION

The average individual weight of harvested prawn was significantly higher in treatment SH (55.45 g), followed by treatments CA (52.83 g) and Co (49.60 g, Table 1). The survival of prawn was also higher in treatment SH (76%) than in treatments CA (66%) and Co (65%). The survival rate of prawn in treatment SH was calculated on the basis of accumulated harvests. The SGR was significantly lower in treatment Co (1.59 %) than in treatments SH (1.68%) and CA (1.64%). The net production of prawn differed significantly among the treatments, with better performance in treatment SH (437 kg/ha), followed by CA (354 kg/ha) and Co (322 kg/ha). FCR was calculated for prawn, which was significantly lower in SH (2.19) than in CA (2.77) and Co (2.95).

Table 1. Comparison of growth and production parameters (mean ± SE, N = 4) of prawn in control (Co), selective harvesting (SH) and claw ablation (CA) treatments during a 137-day culture period.

Species/parameters	Co	SH	CA
Freshwater prawn			
Individual stocking weight (g)	5.63 ± 0.05	5.57 ± 0.07	5.56 ± 0.57[b]
Individual weight at harvest (g)	49.60 ± 0.62[c]	55.45 ± 0.72[a]	5.83 ± 2.44
Survival (%)	65.35 ± 0.52[b]	75.69 ± 0.54[a]	66.32 ± 1.02[b]
SGR (%bw/day)	1.59 ± 0.01[b]	1.68 ± 0.01[a]	11.64 ± 0.01[a]
Gross production (kg/ha)	481 ±10	462 ± 6	471 ± 5
Net production (kg/ha)	445 ± 9	427 ± 6	435 ± 5
Gross production (kg/ha)			
Large (50 g and above)	257 ± 9[b]	407 ± 15[a]	303 ± 20[b]
Medium (33.3–49.9 g)	84 ± 12	84 ± 11	102 ± 16
Small (33.2 g and below)	48 ± 4[a]	13 ± 1b	15 ±1b
Total	389 ± 8[c]	504 ± 6[a]	420 ± 8[b]
Net production (kg/ha)	322 ± 8[c]	437 ± 6[a]	354 ± 8[b]

Mean values with different superscripts indicate a significant difference (P<0.05) based on Tukey's test.
SGR, specific growth rate; bw, body weight.

Individual growth parameters of silver carp and catla and the amount of mola at every harvest did not vary significantly among treatments (Table 2). The combined productions of finfish were also not significantly different among the treatments. However, the combined net productions of all species including freshwater prawn were higher in treatment SH (1244 kg/ha) than those of treatments CA (1161 kg/ha) and Co (1137 kg/ha).

Table 2. Comparison of growth and production parameters (mean ± SE, N = 4) of silver carp and catla in different treatments during a137-day culture period.

Species/parameters	**Co**	**SH**	**CA**
Silver carp			
Individual stocking weight (g)	17.74 ± 0.40	17.66 ± 0.10	18.19 ± 0.29
Individual weight at harvest (g)	240.47 ± 4.86	231.06 ± 2.95	235.68 ± 2.44
Survival (%)	100	100	100
SGR (%bw/day)	1.90 ± 0.01	1.88 ± 0.01	1.87 ± 0.01
Gross production (kg/ha)	481 ±10	462 ± 6	471 ± 5
Net production (kg/ha)	445 ± 9	427 ± 6	435 ± 5
Catla			
Individual stocking weight (g)	22.83 ± 1.14	22.13 ± 0.90	24.05 ± 0.73
Individual weight at harvest (g)	477.27 ± 10.94	466.92 ± 7.19	468.38 ± 16.46
Survival (%)	100	100	100
SGR (%bw/day)	2.22 ± 0.05	2.23 ± 0.02	2.17 ± 0.01
Gross production (kg/ha)	242 ± 2	237 ± 4	238 ± 5
Net production (kg/ha)	231 ± 3	226 ± 4	225 ± 5
Carp combined			
Gross production (kg/ha)	723 ± 10	699 ± 3	709 ± 9
Net production (kg/ha)	676 ± 10	653 ± 3	660 ± 9

SGR, specific growth rate; Co, control; SH, elective harvesting; CA, claw ablation; bw, body weight.

The amount of bi-weekly selectively harvested BC in treatment SH was19% of stocked prawn, which constituted 29% in weight of total harvested prawn. BC individuals in treatment CA were ablated biweekly and released back into the respective ponds, and they constituted 18% of stocked prawn in number. The cumulative selective harvest of BC (2267 individual/ha) and claw-ablated BC (2125 individual/ha) did not vary significantly. The amount of BC at final harvest was significantly higher in treatment Co (1325 per ha) than that in treatments SH (758) and CA (758). However, treatment SH yielded a significantly higher total number of BC (3025 per ha), followed by CA (1741) and Co (1325). Aggregate number of all harvested prawn was also significantly higher in treatment SH (9083 per ha) than in CA (7958) and Co (7841).

The percentages of three different morphotypes among harvested male prawns within each treatment were estimated. Blue claws, orange claws (OC), and small males (SM) were 34.6%, 58.7% and 6.7% respectively in treatment SH; 22.9%, 68.5% and 8.6% in treatment CA; and 17.7%, 55.1% and 27.2% in treatment Co. Some female prawns (4-5% in number) were also recovered at harvest due to error in manual selection of juveniles before stocking. However, the percentage of female prawns did not vary significantly both by number and weight among treatments. Harvested females were of two morphotypes: females with eggs (berried) and females with wide open pleura, indicating that they had already spawned.

The mean weight of BC was significantly higher in Co (85.2 g) than in CA (71.8 g) and SH (68.3 g). The mean weight of OC and SM did not vary significantly among treatments; however, the mean weight of all types of male prawn collectively was significantly higher in SH (55.9 g), followed by CA (53.2 g) and Co (50 g).

Selective harvesting and CA contributed to a 36% and 10% increase in net production of freshwater prawn, respectively, over controls. These differences could have resulted from the survival rate and individual weight differences attained at harvest. While the SGR of prawn between treatments SH and CA did not vary significantly, the survival rate and individual weight at harvest were significantly higher in treatment SH. Interestingly, the numbers of selectively harvested BC in treatment SH and claw-ablated BC in treatment CA were almost similar. Thus, the remaining prawns in both treatments had an equal opportunity to remain free from the growth suppression due to BC (Karplus et al.,1989). However, periodic harvest of BC from treatment SH reduced the prawn density, which also minimized the intra-specific competition for food, space and shelter (Fujimura & Okamoto, 1972), which was not the case in treatments CA and Co. Significantly higher numbers of SM at harvest found in Co indicated that a large number of SM were not able to transform into BC through OC, and their growth was stunted (Karplus et al., 1992) by the suppression phenomenon of BC.

The amounts of prawn and fish harvested are important under the conditions in Bangladesh. Treatment SH yielded 504 kg/ha of prawn, which represented 30% and 20% increased production over Co and CA. This production figure is comparable to other reported values considering the stocking densities and culture period (Kunda et al., 2008; Jose et al., 1992; Nair et al., 1998). Treatment SH had the best FCR (2.2), although this index does not always reflect the direct contribution of diets (D'Abramo & New, 2000), because of natural productivity.

In most polyculture systems, there is a target species and some minor species. The yield of minor species is usually considered to be a bonus to the yield of the target species (Garcia-Perez et al., 2000). Freshwater prawn was the prime species and thus finfish might be considered as secondary species. Although the prawn production (31-37% of total production) was less than finfish production in terms of biomass, its value in sale price was much higher and would constitute about 70-77% of the total benefit in all treatments. This has implications for both profit and household finfish consumption.

Experiment 2: Addition Of Mola Into Polyculture With Freshwater Giant Prawn

INTRODUCTION

Bangladesh is considered one of the most suitable countries in the world for giant freshwater prawn farming, because of its favourable resources and agro climatic condition. The prawn and shrimp sector as a whole is the second largest export industry after readymade garments, generating US$ 380 million annually which is 5.6% of the total value of exports (DoF, 2006). Marine shrimp and freshwater prawn production for 2008-2009 was 145,585 MT. Therefore, this sector had good performance and is gradually changing economic status of the farmers, as well as creating new employment opportunities. But prawn production is low compared to neighboring countries. Males grow larger and faster than females at maturity and, therefore, all-male culture would be economically advantageous (Sagi and Aflalo, 2005). Monosex all male prawn culture may be one means to increase giant freshwater prawn production.

Mola is a very important small indigenous species which is a good source of essential nutrients, particularly vitamin A. Poor farmers in this country are incapable of providing protein rich meals to their children due to high price of commodities, including fish. Culture of freshwater prawn for export market

and mola in the same pond for household consumption may be an innovative option. Introduction of mola will also improve the culture environment by controlling phytoplankton blooms, which are produced mainly due to wastes derived from the high protein diet used in prawn farming (Assaduzzaman et al., 2005). Therefore, the purpose of the proposed study is to develop a new sustainable polyculture technology for all-male giant freshwater prawns and mola to increase the average productivity of high-value prawns for export, as well as to provide highly nutritious fish for household consumption.

MATERIALS AND METHODS

Experiments 2 was done in 15 earthen ponds (9 ponds 100 m^2 each and 6 ponds 140 m^2 each) for 135 days in a completely randomized design into five different treatments with three replications each (Table 3).

Table 3: Experimental design with density of mola and gender difference of prawn as the main variables.

Species	Stocking density/ m^{-2}				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Male prawn	3	-	3	3	3
Female prawn	-	3	-	-	-
Mola	-	-	1	1.5	2

All unwanted fishes were eradicated by rotenone application at 30 g $decimal^{-1}$ (one decimal = 40 m^2) and lime was applied at 250 kg ha^{-1} five days after rotenone application. Urea and TSP were applied at a rate of 50 kg ha^{-1} in each pond after application of lime. Juvenile freshwater prawn (1.75 g) and mola (0.80 g) fry were collected from a nearby nursery in Mymensingh and stocked according to the experimental design.

Formulated feeds were applied at 10% body weight in the first month, reduced to 7% in the second month, then reduced to 3% until the end of the experiment. Feeds were prepared using a local pellet machine with following ingredients: fish meal 15%, mustard oil cake 20%, soybean meal 20%, rice bran 20%, maize flour 20%, molasses 4%, and vitamin-mineral premix 1%.

Water quality parameters were monitored on biweekly basis and growth sampling on monthly basis to adjust the feeding rate and to evaluate fish growth. A number of water quality parameters such as temperature (^{0}C), transparency (cm), pH, dissolved oxygen (mg l^{-1}), alkalinity (mg l^{-1}), phosphate-phosphorus (mg l^{-1}), nitrate-nitrogen (mg l^{-1}), nitrite-nitrogen (mg l^{-1}), ammonia-nitrogen (mg l^{-1}), and chlorophyll-a were measured biweekly. Temperature, transparency, pH and dissolved oxygen were measured in situ and the remaining parameters were measured at the Water Quality and Pond Dynamics Laboratory, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

Prawn and mola were sampled monthly using a seine. Length and weight of 10 individuals of each species from each pond were measured separately to assess health condition and growth. Length was measured using a centimeter scale and weight was taken using a portable balance (OHAUS, model No.CT-1200-S). Culture animals were completely harvested on 17 December 2010 after 135 days of rearing. Partial harvesting was performed by repeated netting, using a seine. Final harvesting was done by draining the ponds using a pump. During harvest, all individuals from each pond were counted and weighed to assess survival rate and pond production.

For the statistical analysis, a one-way ANOVA (Analysis of Variance) was done by using the SPSS (Statistical Package for Social Science) version-11.5. Significance was assigned at the 0.05 level.

RESULTS AND DISCUSSION

The experiment had five treatments: all male prawn + 1 mola m^{-2}, all male prawn + 1.5 mola m^{-2}, all male prawn + 2 mola m^{-2}, only male prawn, and only female prawn, and were treated as T_1, T_2, T_3, T_4 and T_5, respectively with three replications each.

All physical and chemical parameters except transparency and chlorophyll-*a* did not differ significantly among different treatments (Table 4). In treatment T_4, and T_5, where mola was not stocked with prawn, transparency was significantly lower than the other treatments, where prawn was stocked with mola. There were no significant differences ($P<0.05$) among treatments in pH, dissolved oxygen, temperature, alkalinity, NO_3-N, NO_2-N, NH_3-N and PO_4-P. Highest average pH (8.06±0.28) was found in the treatment T_1 and lowest (7.91±0.27) in the treatment T_2. Dissolved oxygen and alkalinity were within a suitable range for prawn culture in all the treatments and did not differ significantly ($P>0.05$) among the treatments. Nitrate, nitrite, ammonia and phosphate did not show significant differences among treatments and were also in suitable range for aquaculture. In treatments T_4 and T_5, prawns were monocultured so that they could not utilize all the nutrients produced in the water body since formulated feed was supplied. On the contrary, in the treatments T_1, T_2, and T_3, mola was stocked with prawn, utilized the phytoplankton produced in the water-body, and transparency was higher in those ponds.

Table 4: Mean values (± SD, N= 33) of water quality parameters for each treatment. Means with the different superscripts are significantly different (*P<0.05*) based on Tukey's test.

Variables	Treatments				
	T_1	T_2	T_3	T_4	T_5
Transparency (cm)	35.73±4.92[a]	36.55±4.06[a]	36.82±4.26[a]	32.64±3.16[b]	31.42±3.37[b]
pH	8.06	7.91	7.95	7.94	7.95
Dissolve oxygen (mg l^{-1})	6.17±0.77	5.99±0.73	5.87±0.67	5.79±0.69	5.78±0.67
Temperature (^{0}C)	29.80±2.18	30.41±1.84	30.08±1.95	29.64±2.01	28.91±3.02
Alkalinity (ppm)	118.09±39.99	118.36±37.65	111.64±34.84	113.27±41.08	113.27±41.08
NO_3-N (mg l^{-1})	0.02±0.02	0.02±0.01	0.02±0.01	0.02±0.02	0.02±0.02
NO_2-N (mg l^{-1})	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
NH_3-N (mg l^{-1})	0.05±0.08	0.06±0.08	0.07±0.09	0.05±0.07	0.05±0.07
PO_4-P (mg l^{-1})	1.68±1.10	1.45±1.21	1.35±0.86	1.45±0.74	1.45±0.74
Chlorophyll-*a* (mg l^{-1})	98.55±39.07[b]	94.48±45.41[b]	97.80±47.10[b]	135.48±61.63[a]	131.77±48.05[a]

Mean harvest weight of female prawns (21.67±1.53) was significantly lower than that of male prawns (Table 5). Mean harvest weight did not vary significantly in all treatments including male prawns. Therefore, female prawns showed lower growth rate than male. Mean weight gain showed similar trends as harvest weight. The rate of survival of male prawns did not differ significantly among treatments with different stocking densities of mola, but differed significantly ($P<0.05$) in monoculture of female and male prawn. At the same time, survival of male and female prawns in monoculture did not differ significantly. Survival of prawns in polyculture was higher than in monoculture. Specific growth rate (SGR) of female prawns was significantly lower treatment in T_5 than the SGR of male prawns either in monoculture or polyculture (Table 5). SGR did not vary significantly in all cultures including male prawns. Net production of prawns varied significantly between monoculture of males and females, but did not vary among different treatments including male prawn. Net production of male prawn monoculture

was significantly (P<0.05) higher than net production of female prawn monoculture. The same trends were shown for gross production.

Mola of same initial weight were stocked in different densities to observe any effect on performance of prawns. There were no significant variations in terms of prawn production among different treatments with male prawns (Figure 1). None of the production characteristics of mola differed significantly among polyculture treatments, mainly due to high variation in mola growth among replicates. There were no significant differences in net production of prawns and mola among treatments, but gross production was highest in polyculture treatments.

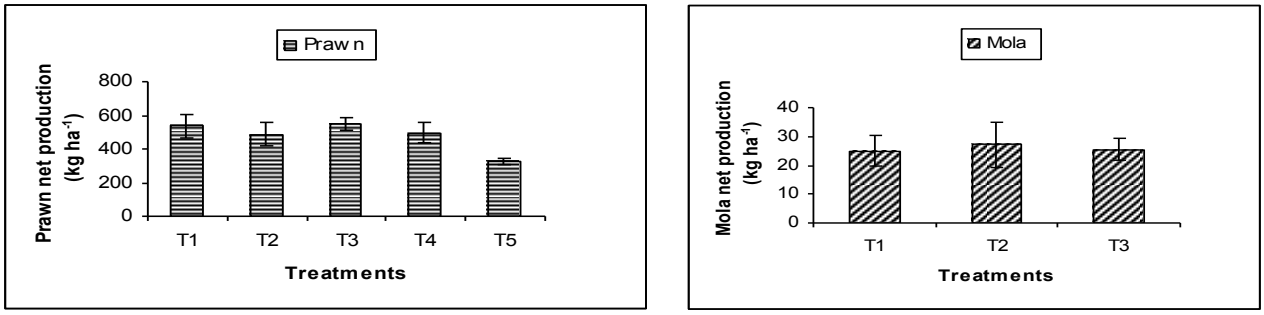


Figure 1. Net production of prawn and mola in different treatments.

Table 5: Production performance of prawn and mola in each treatment. Mean values (± SD,) with the different superscripts are significantly different (*P<0.05*) based on Tukey's test.

Variables	Treatments				
	T_1	T_2	T_3	T_4	T_5
Macrobrachium rosenbergii					
Mean stocking weight	1.75±2.00	1.75±2.64	1.75±1.00	1.75±3.51	1.75±1.53
Mean harvest weight (g)	30.00±3.51^{a}	29.00±2.64^{a}	30.00±1.00^{a}	31.67±2.00^{a}	21.67±1.53^{b}
Mean weight gain (g)	29.92±3.51^{a}	27.25±2.64^{a}	28.25±1.00^{a}	28.25±2.00^{a}	19.92±1.53^{b}
Survival (%)	59.76±1.32^{ab}	59.48±2.77^{ab}	64.78±2.87^{a}	58.51±2.60^{b}	53.91±1.36^{b}
SGR (% body weight)	2.10±0.05^{a}	2.07±0.06^{a}	2.10±0.24^{a}	2.14±0.08^{a}	1.86±0.05^{b}
Net production (Kg h^{-1})	536.62±68.04^{a}	487.63±70.56^{a}	549.19±36.53^{a}	496.89±57.19^{a}	321.71±17.93^{b}
Gross production (Kg h^{-1})	538.05±42.11^{a}	518.86±71.99^{a}	583.20±37.84^{a}	557.63±86.07^{a}	350.01±17.39^{b}
Amblypharyngodon mola					
Mean stocking weight	0.08±0.00	0.08±0.00	0.08±0.00	-	-
Mean harvest weight (g)	3.567±0.71	2.767±0.49	2.800±0.20	-	-
Mean weight gain (g)	2.77±0.71	1.97±0.49	2.00±0.20	-	-
SGR (% body weight)	1.09±0.15	0.91±0.14	0.92±0.05	-	-
Net production (Kg h^{-1})	24.98±5.27	27.12±8.05	25.42±3.83	-	-
Gross production (Kg h^{-1})	32.39±5.92	38.18±8.96	35.56±4.45	-	-
Prawn and mola combined					
Net production (Kg h^{-1})	561.60±69.46^{a}	494.30±64.49^{a}	569.64±52.77^{a}	515.29±70.84^{a}	328.74±25.41^{a}
Gross production (Kg h^{-1})	570.44±41.34^{a}	529.34±63.19^{a}	610.89±60.15^{a}	583.04±105.68^{ab}	360.16±29.55^{b}

Mola mainly feed upon plankton grown in the water-body and prevent plankton blooms in the culture system. Dense growth of phytoplankton can deplete dissolved oxygen in the water-body. Freshwater prawn is an omnivorous species, but mainly a benthivore that utilizes artificial feed. So, a large amount of phytoplankton produced in monoculture ponds remains unutilized and nutrient decomposition associated with this causes heavy growth of phytoplankton, reducing transparency and depleting dissolved oxygen, which is harmful for prawn survival. Mola consume phytoplankton and help to retain better water quality without hampering prawn growth or competing with prawn for feed.

Addition of mola at different densities had no effect on survival, gain in weight, and production performance of prawn. Therefore, mola may be stocked as an additional species with freshwater prawn that would give higher total gross production, provide family nutrition as well as generate additional income. Freshwater prawn polyculture with mola has good potential to be implemented in Bangladesh and can be a sustainable way for income generation, as well as a good source of vitamin A. Moreover, water quality management will be easier and good pond ecology more sustainable for production of freshwater prawn.

Experiment 3: Effects of Addition of Carps on Growth, Size Structure and Production of Prawn and Mola in A Polyculture System

INTRODUCTION

Several methods have been employed to increase per unit production (kg/ha) of prawns, including increased size at stocking, increased stocking densities, grading animals prior to stocking and selective harvesting of the largest animals during the growing season, but it is still a challenge is to develop a technology that can raise pond productivity in a sustainable way, while minimizing the use of inputs like energy and capital.

The farmers of Bangladesh are incapable of providing protein rich meal to their children due to the higher price. Zafri and Ahmed (1981) reported that, mola contains 200 IU of vitamin-A per gram of edible protein. A medium size mola individual has about 2.0 g of edible protein in its muscles, which contain 400 IU of vitamin-A. This means that if four mola are eaten daily, it may provide more than 1500 IU of vitamin-A, sufficient to save children from night blindness caused by deficiency of vitamin-A. Introduction of mola also improves the culture environment by reducing phytoplankton blooms, which are mainly due to waste derived from high protein diet used in prawn farming (Assaduzzaman et al., 2005).

Aquaculture in Bangladesh has been developed through the culture of large carps of both native (e.g., rohu *(Labeo rohita)*, catla, and mrigal *Cirrhinus mrigala* and exotic (e.g., silver, grass *Ctenopharyngodon idella*, and common carp *Cyprinus carpio* origin. Several experiments have been carried out to evaluate any interference in the pond among feeding fish (Alim et al., 2004; Milstein et al., 2002; Wahab et al., 2001, 2002, 2003). Silver carp is expected to have a strong impact on the pond ecology because it is a very effective filter feeder and releases nutrients as feces (Milstein, 1992; Milstein et al., 1985). It also is significant to the farmer's family nutrition as it is a cheap fish that a family can afford to eat instead of selling. It is also easily accessible to the poorer section of the population because of its low market price. Among the Chinese carps, silver carp is popular due to its fast growth and unique food habits. They are mainly phytoplankton feeders and can reduce algal blooms. Catla is the fastest growing fish among the three Indian major carps and a suitable species for polyculture. It has also a good market value.

Development of appropriate culture technologies is one of the most important issues in Bangladesh to contribute to the national economy and to improve the standard of living of people through production of freshwater prawn along with high valued carps and nutrient rich small fish. Polyculture may produce increased yield if fish with different feeding habits are stocked in proper ratios and combinations (Halver, 1984). To address this issue, this study was designed to investigate impacts of including carps on growth, production, and pond ecosystem in freshwater prawn-carp-mola polyculture systems.

In order to develop prawn-carp-mola polyculture technology and to enhance overall production and economic benefit, the following objectives were set out for the proposed research: 1. To evaluate the effects of inclusion of catla and silver carp on growth and production of prawn and mola; and 2. To evaluate the effects of addition of carps on pond ecology, prawn-mola production and overall production in the system.

MATERIALS AND METHODS

The experiment was carried out at the Fisheries Field Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh in 15 earthen pond (all ponds are of 140 m^2 each) for a period of 70 days in a completely randomized design into five different treatments with three replications of each (Table 6).

Table 6: Stocking densities of animals in each treatment. To maintain same total biomass, mola stocking density varied among treatments.

Species	Treatment (Stocking density per 40m^2)				
	T_1	T_2	T_3	T_4	T_5
Prawn	120	120	120	120	-
Mola	83	60	60	60	272
Catla	-	6	-	2	-
Silver carp	-	-	6	4	-

All unwanted fishes were eradicated by drying the pond, and lime was applied at 250 kg ha^{-1} five days after drying. Urea and TSP were applied at 50 kg ha^{-1} in each pond after application of lime. The average weight of freshwater prawn juveniles, mola, catla, and silver carp were 2.3, 1.5, 20.94, 20.82 g. They were collected from a nearby nursery in Mymensingh and stocked according to the experimental design. Stocking densities of mola were different in the treatments T_1 and T_5 in order to maintain comparable fish biomass among treatments.

Formulated feeds were applied at 10% body weight of prawn for the first month, reduced to 7% for the second month, then reduced to 5% until the end of the experiment. Feeds were prepared using a local pellet machine with following ingredients: Fish meal 15%, Mustard oil cake 20%, Soybean meal 20%, Rice bran 20%, Maize flour 20%, Molasses 4%, Vitamin-mineral premix 1%.

A number of water quality parameters, including temperature (^{0}C), transparency (cm), pH, dissolved oxygen (mg l^{-1}), alkalinity (mg l^{-1}), phosphate-phosphorus (mg l^{-1}), nitrate-nitrogen (mg l^{-1}), nitrite-nitrogen (mg l^{-1}), ammonia-nitrogen (mg l^{-1}), and chlorophyll-a were measured biweekly. Temperature, transparency, pH, and dissolved oxygen were measured on site and the remaining parameters were measured at the Water Quality and Pond Dynamics Laboratory, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

Prawn, mola, catla and silver carp were sampled monthly using a seine. Length and weight of 10 animals of each species from each pond were measured separately to assess growth and to adjust feeding rate.

Length was measured by using a centimeter scale and weight was taken by using a portable balance (OHAUS, model No.CT-1200-S). Animals were completely harvested on 10-12 September 2011 after 70 days of rearing. Partial harvesting was performed by repeated netting, using a seine. Final harvest was done by draining the ponds using a pump. During harvesting, all animals in each pond were counted and weighed individually to assess survival rate and pond production.

For statistical analysis of the data, one-way ANOVA (Analysis of Variance) was done by using the SPSS (Statistical Package for Social Science) version-11.5. Significance was assigned at the 0.05% level.

RESULTS

The experiment had five treatments: prawn-mola, prawn-mola-catla, prawn-mola-silver carp, prawn-mola-catla-silver carp, and only mola, and were redorded as T_1, T_2, T_3, T_4 and T_5, respectively with three replications each.

All physical and chemical parameters, except transparency did not differ significantly among treatment (Table 7). Water temperature was about 2.8 ºC and transparency about 32 cm. In treatments T_3, where silver carp was stocked with prawn and mola, transparency was significantly higher than the treatments without silver carp (Table 7, Figure 2). pH was approximately 8.4, DO about 6.1 mg l^{-1}, and alkalinity 130 mg l^{-1}. Nitrate concentrations were about 0.02 mg l^{-1}, NO_2-N about 0.003 mg l^{-1}, NH_3-N about 0.03 mg l^{-1}, PO_4-P about 1.7 mg l^{-1}, and chlorophyll-*a* about 130 µg l^{-1}.

Table 7: Mean values (± SD, N= 18) of water quality parameters of different treatments. Means with the different superscripts are significantly different (*P<0.05*) based on Duncan's test.

	Treatments				
Variables	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Transparency (cm)	32.83±3.79^{b} (28-42)	32.33±3.28^{bc} (28-37)	36.67±3.64^{a} (30-42)	33.11±3.23^{b} (26-38)	30.44±1.85^{c} (25-33)
pH	8.42 (8-8.8)	8.36 (7.9-8.9)	8.31 (7.9-8.8)	8.35 (8.0-9.2)	8.4 (8.1-9.0)
DO (mg/l)	6.17±0.77 (4.64-7.32)	6.11±0.76 (4.61-7.72)	6.11±0.84 (4.65-7.72)	6.02±0.85 (4.01-7.01)	6.09±0.80 (4.58-7.73)
Temperature (°C)	28.01±2.87 (24.2-33.7)	28.19±2.77 (24.6-33.9)	28.41±2.95 (24.9-33.9)	28.47±2.93 (25.2-33.9)	28.41±3.04 (24.6-34.0)
Alkalinity (mg l^{-1})	137.16±30.05 (66-182)	130.27±33.11 (78-178)	134.05±36.34 (66-220)	129.16±28.40 (64-172)	134.27±34.18 (68-188)
NO_3-N (mg l^{-1})	0.02±0.01 (0.00-0.05)	0.02±0.01 (0.00-0.06)	0.02±0.02 (0.00-0.07)	0.02±0.01 (0.00-0.06)	0.03±0.02 (0.00-0.10)
NO_2-N (mg l^{-1})	0.0032±0.0028 (0.00-0.01)	0.0035±0.0032(0.00-0.01)	0.0038±0.0036 (0.00-0.01)	0.0035±0.0030 (0.00-0.01)	0.0035±0.0030 (0.00-0.01)
NH_3-N (mg l^{-1})	0.03±0.05 (0.00-0.16)	0.02±0.04 (0.00-0.14)	0.03±0.07 (0.00-0.25)	0.04±0.08 (0.00-0.34)	0.04±0.06 (0.00-0.18)
PO_4-P (mg l^{-1})	1.75±1.14 (0.27-3.94)	1.51±0.92 (0.32-3.26)	1.74±1.12 (0.27-3.38)	1.66±1.14 (0.41-3.56)	1.77±1.17 (0.29-4.16)
Chlorophyll-*a* (µg l^{-1})	145.11±65.70 (54.74-270.13)	134.13±61.22 (44.03-272.51)	127.74±69.82 (34.51-318.92)	118.86±61.13 (38.08-223.72)	141.47±59.98 (73.78-252.28)

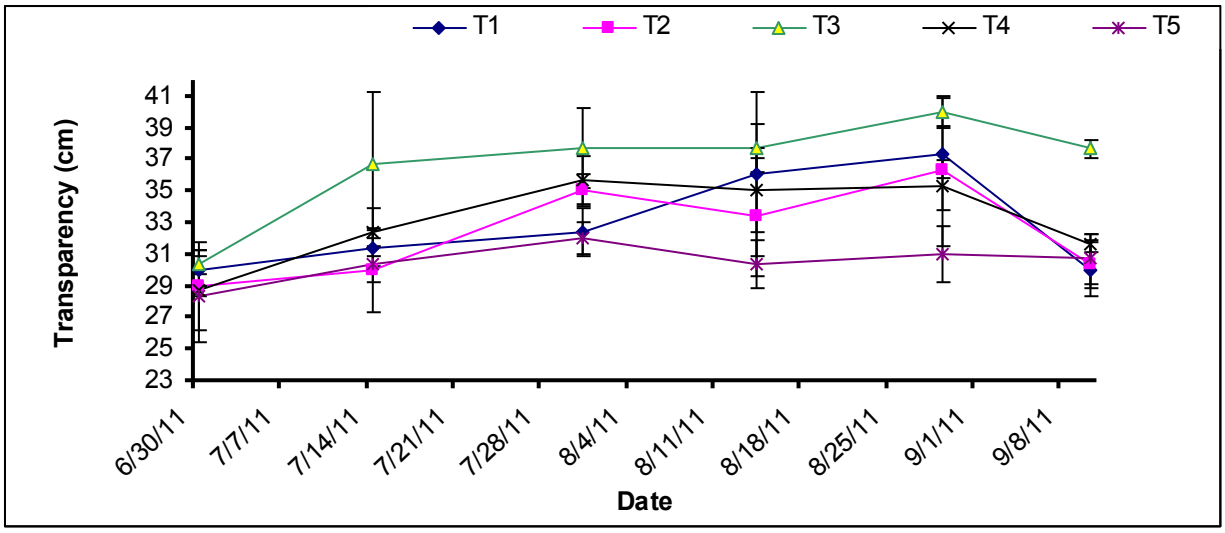


Figure 2. Variation in transparency among treatments.

There were no significant differences in harvested weight, weight gain, or specific growth rate of freshwater prawns in any treatment (Table 8). However, net and gross productions of prawn were significantly higher in T_3 and T_4, than in $T_1$and T_2, indicating that polyculture with silver carp improved prawn yields.

Survival, weight gain, and daily growth rate of mola did not differ significantly among different treatments. The net and gross production of mola was significantly higher in T_5 than in other treatments. This was the result of a much higher stocking density of mola in monoculture (T_5) compared to polyculture.

The survival rates of carps were not significantly different among treatments (Table 8). However, average final weight, weight gain, and daily growth rate of carps were significantly lower in T_3 (silver carp-prawn-mola) than T_2 (prawn-mola-catla) and T_4 (prawn-mola-catla-silver carp), indicating that production performance of silver carp alone was lower than that of catla alone, or silver carp with catla. Both net and gross production were again significantly higher in the treatment T_2 and T_4 than in T_3.

As the experiment was designed on the basis of same biomass among different treatments, then total net production can be a good indicator to evaluate production performance among different combination of species. Total net production and total gross production were significantly higher in polyculture treatments T_2 and T_4 with catla or silver carp-catla compared to other treatments.

Table 8. Production performance of prawn, mola, and carps in each treatment. Mean values (± SD,) with the different superscripts in the same rows were significantly different (P<0.05) based on Duncan's test.

Species	Characters	Treatment					Level of significance
		T_1	T_2	T_3	T_4	T_5	
Prawn	Survival (%)	34.92±7.27	37.06±6.88	55.39±14.92	56.34±13.87		NS
	Average final weight (g)	12.65±2.09	12.59±1.45	13.18±1.03	12.16±2.17		NS
	Average weight gain (%)	450.29±91.03	447.68±63.21	473.33±44.95	428.99±94.64		NS
	SGR (% bw day^{-1})	2.42±.25	2.42±.17	2.49±.11	2.36±.25		NS
	Net Production (kg ha^{-1})	65.09±40.77^{b}	72.87±41.07^{b}	147.38±42.11^{a}	131.47±24.35^{ab}		*
	Gross production(kg/ha)	134.09±40.77^{b}	141.87±41.07^{b}	216.39±42.12^{a}	200.47±24.35^{ab}		*
Carps	Survival (%)		85.71±4.76	92.06±9.91	88.89±5.49		NS
	Average stocking wt (gm)		20.83±0.35	20.97±0.63	20.92±0.04		NS
	Average final wt (gm)		440.47±14.00^{a}	188.32±4.15^{b}	378.69±108.80^{a}		*
	Average weight gain (%)		2014.40±64.65^{a}	798.99±42.53^{b}	1710.23±520.30^{a}		*
	SGR (% bw day $^{-1}$)		4.35 ±0.04^{a}	3.13±0.06^{b}	4.10±0.38^{a}		*
	Net Production (kg ha^{-1})		534.80±29.32^{a}	229.01±33.96^{b}	470.08±126.98^{a}		*
	Gross production (kg ha^{-1})		566.5±28.86^{a}	260.45±33.19^{b}	501.46±126.99^{a}		*
Mola	Stocking no.	290	210	210	210	950	
	Average harvest no.	410.00±60	326.67±66.58	304±75.29	256.67±70.95	1317.33±116.79	NS
	Average final wt (gm)	1.54±0.084	1.70±0.23	2.11±0.50	2.38±1.36	1.46±0.02	NS
	Average weight gain (%)	2.44±5.59	13.11±15.35	40.67±33.60	58.44±91.03	-2.66±1.33	NS
	Net Production (kg ha^{-1})	13.2±4.48^{b}	16.72±6.58^{b}	21.7±4.73^{ab}	16.65±10.11^{b}	35.57±12.01^{a}	*
	Gross production (kg ha^{-1})	44.79±4.48^{b}	39.22±6.58^{b}	44.29±4.73^{b}	39.16±10.11^{b}	137.36±12.01^{a}	*
Combined	Net Production (kg ha^{-1})	77.16±44.71^{c}	622.74±46.81^{a}	396.54±62.57^{b}	616.56±146.36^{a}	35.57±12.01^{c}	*
	Gross production (kg ha^{-1})	178.88±44.71^{c}	747.14±46.71^{a}	521.13±61.63^{b}	741.09±146.37^{a}	137.36±12.01^{a}	*

DISCUSSION

Suitable ranges of all water quality parameters are very important for fish culture, as they provide good environment for health, growth, and development of fish food. The ranges of water temperature in this study were similar to those recorded from rice field culture by Uddin (1998) and Das (2002), who found maximum carp production at those temperatures. pH was similar to values observed to values by Whitton et al. (1988), and dissolved oxygen to values observed by Uddin (1998) and Nirod (1997), again resulting in rapid growth. Similarly, NH_3 values were within acceptable ranges for goods survival growth.

Transparency ranged from 25-42 cm in the present study which was within the recommended optimum range 15-40 cm suggested by Boyd (1992). Significantly higher transparency in treatment T_3 was probably due to stocking silver carp. The silver carp is a phytoplanktivorous filter-feeder, consuming algae of various sizes (Cremer and Smitherman, 1980; Smith, 1989). It is an appropriate species for polyculture with freshwater prawn and other carps (Rahman, 2010). The fast-growing silver carp increased grazing pressure on phytoplankton, which increased water transparency. Catla consumes zooplankton that collects in open waters (Natarajan and Jhingran, 1961), while mola consume phytoplankton (Jhingran and Pullin, 1985). The number of phytoplankton increased dramatically in the absence of silver carp in ponds supplied with fertilizer, and at the same time, catla consume zooplankton to reduce grazing pressure on phytoplankton.

In the present study, average harvested weight of prawn was comparable with the findings of Karim (2001) for 70 days of culture. Average weight gain and SGR of prawn did not differ significantly among the treatments. Net and gross production values were comparatively lower than Lan et al. (2006) who reported net production at 194-373 kg h^{-1}. Net and gross production of prawn were significantly higher with silver carp and with silver carp-catla, but not with mola and mola-catla, probably due to the grazing interactions indicated above. Inclusion of carps did not harm the production of prawn, but maintained higher water quality. Milstein (1992) stated that silver carp can also raise benthophagous fish food resources through faecal pellets in polyculture systems.

Different combinations of carp species with prawn and mola did not show significant differences among treatments in terms of survival of carps. Gross production of catla and silver carp ranged from 260-566 kg h^{-1} in 70 days, which were lower than annual finfish production of 660 kg h^{-1} mentioned as expected by Asaduzzaman et al. (2006a). Average weight gain, SGR, net and gross production of all carps combined were significantly lower for silver carp alone than catla alone or catla-silver carp. Lack of a zooplankton feeding fish in the cultures without catla may have limited overall carp production in T_3. Balanced stocking densities of prawn, mola, catla and silver carp appeared to develop a synergistic interaction (Milstein, 2005) that resulted in net and gross production increases.

Average final weight and average weight gain of mola did not differ significantly among treatments as stocking density was adjusted to achieve the same biomass. Average weight gain was negative in treatment T_5, as mola offspring of smaller size were found during harvesting. Net and gross productions were significantly higher in treatment T_5 in which only mola was stocked in higher densities. But no significant difference was found in mola production with prawn, prawn-catla, prawn-silver carp, and prawn-catla-silver carp indicating there were no negative effects of polycultures on the growth and production of mola.

Net and gross productions were significantly higher when prawn and mola were stocked with catla or with catla-silver carp than with only silver carp or without carps. The lowest production was found by stocking only mola indicating mola was unable to utilize all of the water column and cannot be an economic monoculture system.

Freshwater prawn in polyculture with carps and mola have good potential to be implemented in Bangladesh. It can be a sustainable way for income generation due to high production as well as a good source of protein. Moreover, water quality management will be easier and good pond ecology can be assured. Above all, the farmers will make higher profit with prawn and carp and be able to provide nutrition to family members by inclusion of small indigenous fish, mola.

LITERATURE CITED

M A Alim, M.A., M.A. Wahab, and A. Milstein, 2004. Effects of adding different proportions of the small fish punti (*Puntius sophore*) and mola (*Amblypharyngodon mola*) to a polyculture of large carp. Aquaculture Research, 35:124-133.

Asaduzzaman, M., M.A. Wahab, A. Kadir, A.S.M. Kibria, and M. Kunda, 2005. Observation on the some of the environmental parameters and feed quality of selected giant freshwater prawn *Macrobrachium rosenbergii* farms in Bangladesh. Bangladesh Journal of Fisheries Research, 9:112-122.

Asaduzzaman, M., M.A. Wahab, Y. Yi, and J.S. Diana, 2006a. Bangladesh prawn-farming survey reports industry evolution. Global Aquaculture Advocate, 9:41-43.

Asaduzzaman, M., M. A. Islam, M. Kunda, M. S. Uddin, M.A. Wahab, 2006b. Performance of over-wintering juveniles of giant freshwater prawn under monosex and mixed sex culture systems. Progressive Agriculture, 17:212-223.

Boyd, C.E., 1992. Water quality management for pond fish culture. Elsevier Science Publishers B. V., Amsterdam, the Netherlands, 318 p.

Cremer, M., and R.O. Smitheran. 1980. Food habits and growth of silver and bighead carp in cages and ponds. Aquaculture, 20:57-64.

D'Abramo L.R. and M. B. New. 2000. Nutrition, feeds and feeding. In: Freshwater Prawn Culture. M.B. New and W.C. Valenti (Editors), Blackwell Science, Oxford, UK, pp. 203-220.

Das, B.C., 2002. Studies on the effects of stocking density on growth, survival and yield of *Amblypharyngodon mola* and the performance of *Rohtee cotio* in combination with *Barbodes gonionotus* and with *Cyprinus carpio* in rice fields. M.Sc. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, 113 p.

DoF (Department of Fisheries), 2006. Fishery Statistical Yearbook of Bangladesh, 2005-2006. Fisheries Resources Survey System, Department of Fisheries, Bangladesh, Dhaka.

Fujimura, T., and H. Okamoto. 1972. Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. In: Coastal Aquaculture in the Indo-Pacific Region, T.V.R. Pillay (Editor),. Fishing News Books, Blackwell Science, Oxford, UK, pp. 313-327.

Garcia-Perez A., D.E. Alston, and R. Cortes-Maldonado. 2000. Growth, survival, yield and size distributions of freshwater prawn *Macrobrachium rosenbergii* and tilapia *Oreochromis niloticus* in polyculture and monoculture systems in Puerto Rico. Journal of World Aquaculture Society, 31: 446-451.

Halver, J. E., 1984. Special methods in pond fish husbandry. Akademiai Nyomda, Budapest, 146 p.

Jhingran, V.G., and R.S.V. Pullin, 1985. A Hatchery Manual for the Common, Chinese and Indian Major Carp. ICLARM Contribution, ADB & ICLARM Publication. Vol. 252. 191 p.

Jose S., C.M. Nair, and D.M. Thampy. 1992. Culture of *Macrobrachium rosenbergii* in homestead ponds. In: Freshwater Prawns. Proceedings of the National Symposium of Freshwater Prawns (*Macrobrachium* spp.). E.G. Silas (Editor), Kerala Agricultural University, Thrissur, India, pp. 247-250.

Karim, M., 2001. Report on Feasibility of Early Shrimp Farming with Over-Wintered Galda Juveniles. p. 13.

Karplus, I., E. Samsonov, G. Hulata, and A. Milstein. 1989. Social control of growth in *Macrobrachium rosenbergii*. I. The effect of claw ablation on survival and growth of communally raised prawns. Aquaculture, 80:325-355.

Karplus, I., G. Hulata, D. Ovadia, and R. Jaffe. 1992. Social control of growth in *Macrobrachium rosenbergii*. III. The role of claws in bull-runt interactions. Aquaculture, 105:281- 296.

Kunda M., M.E. Azim, M.A. Wahab, S. Dewan, N. Roos, and S.H. Thilsted. 2008. Potential of mixed culture of freshwater prawn (*Macrobrachium rosenbergii*) and self-recruiting small fish mola (*Amblypharyngodon mola*) in rotational rice-fish/prawn culture systems in Bangladesh. Aquaculture Research, 39:506-517.

Lan, L.M., D.N. Long, and J. Micha, 2006. The effects of densities and feed types on the production of *Macrobrachium rosenbergii* (de Man) in the rotational rice-prawn system. Aquaculture Research, 37:1297-1304.

Milstein, A., 1992. Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia, 231:177-186.

Milstein, A., 2005. Effect of periphyton on water quality. In: Azim, M.E., M.C.J. Verdegem, A.A. van Dam, and M.C.J. Biveridge (Editors), Periphyton, Ecology, Exploitation and Management. CABI Publishing, Wallingford, U.K., pp. 179-190.

Milstein, A., and B. Hepher, 1985. Principal component analysis of interaction between fish species and the ecological conditions in the fish ponds: I. Phytoplankton. Aquaculture and Fisheries Management, 16: 305-317.

Milstein, A., M.A. Wahab, and M.M. Rahman, 2002. The effects of common carp, *Cyprinus carpio* (L.) and mrigal, *Cirrhinus mirgala* (Hamilton) as bottom feeders in major Indian carp polyculture. Aquaculture Research, 33: 547-556.

Nair K.G.R., S. Jose, P.T. Mathew, and J. Stephen. 1998. Growth, survival and production of *Macrobrachium rosenbergii* (De Man) with an indigenous feed. In: Proceedings of the National Symposium on Technological Advancement in Fisheries and its Impact on Rural Development. M.S. Hameed and B.M. Kurup (Editors), CUSAT, Cochin, India, pp. 135–140.

Natarajan A.V., Jhingran A.G., 1961. Index of preponderance – A method of grading the food elements in the stomach analysis of fishes. Indian Journal of Fisheries, 8:54-59.

Nirod, D.B., 1997. Effect on stocking density on the growth and production of mola (*Amblypharyngodon mola*). M.S. Thesis, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. 75 p.

Rahman, S.M.S, M.A. Wahab, M. Kunda, M.A. Islam, and M.E. Azim, 2010b. Density effects of silver carp *Hypophthalmichthys molitrix* and catla *Catla catla* on the production system in all-male freshwater prawn-finfish polyculture ponds. Aquaculture Research, 41:456-466.

Sagi A. and E.D. Aflalo, 2005. The androgenic gland and monosex culture of freshwater prawn Macrobrachium rosenbergii (De Man): a biotechnological perspective. Aquaculture Research 36,:231-237.

Smith, D.W., 1989. The feeding selectivity of silver carp, *Hypophthalmicthys molitrix* Val. Journal Fish Biology, 34:819-828.

Uddin, M.J., 1998. Effects of fish culture in rice fields on the yields of rice and nutrients availability in soil, straw and grain. M.S. Thesis, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, 115 p.

Wahab, M.A., M.A. Alim, and A. Milstein, 2003. Effects of adding the small fish punti (*Puntius sophore*) and/or mola (*Amblypharyngodon mola*) to a polyculture of large carp. Aquaculture Research, 34:149-164.

Wahab, M.A., M.M. Rahman, and A. Milstein, 2002. The effect of common carp *Cyprinus carpio* (L) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research, 33:547-557.

Wahab, M. A., M.M. Rahman. and A. Milstein, 2001. The effect of common carp *Cyprinus carpio* and mrigal *Cirrhinus mrigala* as bottom feeders in Indian carp polyculture. World Aquaculture, 32:50-52 & 69.

Whitton, B.A., A. Aziz, P. Franeis, J.A. Rother, T.W. Simon, and Z.N. Tahmida, 1988. Ecology of deep water rice in Bangladesh (physical and chemical environment). Hydrobiologia, 169:3-67.

Zafri, A. and K. Ahmed, 1981. Studies on the vitamin-a content of fresh water fishes, content and distribution of vitamin-A in mola (*Amblypharyngodon mola*) and dhela (*Rohtee cotio)*. Bangladesh Journal of Biological Sciences, 10:47-53.

Evaluation of Performance of Different Tilapia Species

Quality Seedstock Development/Experiment/09QSD04PU

Sebastian W. Chenyambuga, Ester Meiluidie, Nazael Madalla and
Berno V. Mnembuka
Sokoine University of Agriculture
Morogoro, Tanzania.

Rebecca Lochmann
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

ABSTRACT

A growth trial was carried out for 90 days to compare the growth performance and survival rate of Nile tilapia (*Oreochromis niloticus)* and Jipe tilapia (*Oreochromis jipe*), Wami tilapia (*Oreochromis urolepis hornorum)* and Ruvuma tilapia *(Oreochromis ruvumae)*.. The study was conducted on-station at Sokoine University of Agriculture (SUA) and on-farm in ponds of small-scale fish farmers at Mkuyuni, Morogoro, Tanzania. In the on-farm experiment, the mean final weight, weight gain, growth rate, final length and final width differed significantly ($P<0.001$) among the species. Nile tilapia (*O. niloticus*) gained more weight (61.3g) than *O. hornorum* (35.3g) *and O. ruvumae* (13.8g). The final weight, length and width of *O. niloticus* exceeded that of *O. hornorum* by 26.2g, 2.4cm and 0.7cm, respectively. The mean final weight, length and width of *O. hornorum* were higher than those of *O. ruvumae* by 23.6g, 2.7cm and 1cm, respectively.

The results from the on-station experiment showed that there was no significant ($P>0.05$) difference between *O. niloticus* and *O. hornorum*, but the two species differed significantly ($P<0.01$) from *O. ruvumae* and *O. jipe*. The *O. niloticus* had the highest weight gain (24.2 g), growth rate (0.3g/d), final length (11.5cm) and final width (3.3cm) and it was followed by *O. hornorum.* The *O. ruvumae* and *O. jipe* showed poor performance in all parameters. The growth performances of *O. niloticus* and *O. hornorum* were significantly ($P<0.05$) higher in the on-farm experiment than in the on-station experiment, but that of *O. ruvumae* were not significantly ($P\geq0.05$) different between the two locations. The highest survival rates (85.6% (on-farm) and 100% (on-station)) were observed on *O. niloticus*, followed by *O. ruvumae*. *Oreochromis urolepis hornorum* had the lowest survival rate (63.5 – 66.7%) in both experiments. For all species, fish reared at the on-station showed higher survival rate (66.7 – 100%) compared to those reared in farmers' ponds (63.5 – 85.6%).

The results for chemical composition of the fish bodies indicated that the species did not differ significantly in dry matter (DM) and ash contents, but differed significantly ($P<0.05$) in crude protein (CP) and fat (EE) contents for the on-farm experiment. *Oreochromis urolepis hornorum* had the highest CP content (58.09%) and EE (30.12%) while *O. niloticus* had the lowest values (52.23% CP and 16.83% EE). For the on-station experiment, the DM, CP and ash contents of the species were not significantly ($P\geq0.05$) different. It is concluded that *Oreochromis niloticus* is superior to *Oreochromis urolepis hornorum, Oreochromis jipe*, and *Oreochromis ruvumae* in terms of growth performance and survival rate.

INTRODUCTION

Aquaculture in Tanzania started in the 1950's with the pond culture of the tilapia species native to the region, including Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*) and Zanzibar tilapia (*Oreochromis urolepis hornorum*) (Rice *et al*., 2006). Other species which have been used commercially in Aquaculture include *O. urolepis hornorum* originating from the Wami river of north-central Tanzania and *O. karongae* native to Lake Nyasa (Lake Malawi). At the moment, more than 95% of the farmers culture Nile tilapia (*Oreochromis niloticus*) in earthen ponds under mixed-sex culture (Kaliba *et al*., 2006). Pond culture of Nile tilapia is now viewed as a possible source of livelihood for farmers residing in proximity to the urban markets of cities and towns. The emphasis of the national fisheries policy (URT, 1997) is on a semi-intensive integrated mode of fish culture, focusing on Nile tilapia. The Nile tilapia is given first priority due to their better characteristics that include fast growth, short food chain, efficient conversion of food, high fecundity (which provides opportunity for distribution of fingerlings from farmer to farmer), tolerance to a wide range of environmental parameters, and good product quality (Hussain *et al*., 2000; Neves *et al*., 2008).

In Tanzania, fish farmers obtain fingerlings from government fry centres and fisheries institutes. Some fish farmers produce their own fingerlings and sell them to other farmers. Because of the lack of controlled breeding, most ponds in the country are yielding only small-sized tilapia and production of fish is not encouraging. Quite a number of farmers feel that their fish are small due to stunted growth, and this is discouraging them from continuing with fish farming operations. Therefore, there is a need for bio-prospecting for various species of tilapia to identify the species suitable for aquaculture in Tanzania. Because Tanzania is a region with very high natural tilapiine fish diversity (Rice *et al*., 2006), the ability to tap into the diverse natural pool of tilapiine fish genes is very important. This study was intended to evaluate the productive performance (growth rate, feed conversion ratio and market body weight) of different species of tilapia.

The objectives of the study were;

1. To compare the performance (growth rate, survival, feed conversion ratio and mature body size) of different species of Nile tilapia
2. To carry out economic analysis of raising the different species
3. To determine the management requirements of the best tilapia species identified under objectives one and two.
4. To train farmers on the proper methods to culture the improved tilapia species.

MATERIALS AND METHODS

Study location and experimental Fish

Growth performances of Nile tilapia (*Oreochromis niloticus)*, Jipe tilapia (*Oreochromis jipe*), Wami tilapia (*Oreochromis urolepis hornorum)* and Ruvuma tilapia *(Oreochromis ruvumae)* were studied. The study was undertaken on-station in ponds at Sokoine University of Agriculture (SUA) and on-farm in ponds of small-scale fish farmers at Chang'a and Kibwaya villages in Mkuyuni division, Morogoro rural district, Tanzania. Nile tilapia (*Oreochromis niloticus*) fingerlings were collected from Kingolwira Fish Farming Centre. The fingerlings of *Oreochromis jipe* were collected from Lake Jipe in Mwanga district while *Oreochromis urolepis hornorum* and *Oreochromis ruvumae* were collected from river Wami at Dakawa sub-town, Mvomero district and river Ruvuma at Litapwasi village, Songea district, respectively. The fingerlings were collected from their respective sources and brought to SUA. At SUA the fingerlings of each species were kept separately in concrete tanks prior to the start of the experiment**.**

Experimental procedure

The experiment was conducted for 90 days, from April to July 2011. For the on–station experiment, two

earthen ponds (300 m^2 each) were used and each pond was fitted with four hapas of 6 m^2 surface area and one meter depth each. In total eight hapas were used and each tilapia species was allocated to one hapa in each pond at random. Prior to commencement of the experiment the ponds were drained, cleaned and allowed to dry for one week. Then the hapas were set and the ponds were refilled with water and poultry manure was added at a rate of 7.5 kg per pond. Stocking density was 2 fingerlings per m^2 in each hapa. All Fish in the hapas were supplemented daily with concentrate comprised of soybean meal (40%), maize bran (59%) and mineral (1%). The concentrate diet was provided at a level of 10 %, 7% and 5% of fish biomass during the first month, second month and third month of the experiment, respectively. Body weights of fish were measured using a digital weighing balance at the start of the experiment and then at monthly intervals for a period of 90 days. Similarly body length and width were measured at the beginning of the experiment and then at monthly intervals by using a measuring board with a ruler. Water quality parameters were measured at weekly intervals. Temperature and dissolved oxygen (DO) were measured by using YSI 55 instrument; water pH, nitrate, nitrite by using JBL Easy Test strips and water transparency by using secchi disk. The experiment was completed on 20th July 2011.

For the on–farm experiment, a total of six farmers from Chang'a and Kibwaya villages participated in the experiment. Each species of tilapia was distributed to two different farmers, making two replications for each species. The pond size varied from farmer to farmer and ranged between 50 m^2 and 200 m^2. The ponds were filled with water by using channels available in the villages and fertilized by using farm yard manure. The fingerlings were stocked at a density of 2 fish per m^2 in each pond. The farmers provided supplementary feeds to their fish. The supplementary feeds included maize bran, vegetables and kitchen left overs that were obtained within the farmers' homesteads. Body measurements (weight, length and width) of fish and water quality parameters were measured as in the on-station experiment, at the start of the experiment and then at monthly intervals. The experiment was completed on 19th July 2011.

In both experiments growth parameters that were determined included body weight gain (W_1- W_0) and average daily body weight gain (W_1 -W_0)/t, expressed as weight gain per fish per day, whereby W_0 and W_1 are initial weight and final weight, respectively, and t is time interval in days. Survival rate was calculated as ((N- D)/N) x 100, where by N is total number of fish stocked and D is number of fish died.

The profitability of raising each species was computed using Gross Margin Analysis as follows:

GM = TR – TVC

Where:
GM = Gross margin
TR = Total revenue (Sales from fish)
TVC = Total variable costs (Costs of feeds, fingerlings, transport and labour)

Data analysis

The data were analyzed using the General Linear Model (GLM) of the Statistical Analysis System (SAS 1998) software. The effect of species on body weight, body length, body width and growth rate of the fish was tested. Factors tested were species, location and their interactions. Initial body measurements were used as a covariate during the analysis of growth data. Descriptive statistics were generated for water quality parameters. The Chi-Square (χ^2) test was used to assess the effect of species on survival rate of the fish.

RESULTS

The water quality parameters that were measured in this study include water temperature, Dissolved oxygen, pH, nitrite and nitrate and their mean values during the experimental period are shown in Table 6.

The results show that water pH and transparency differed significantly (P<0.01) between on - farm and on – station, but there were no significant differences between on-station and on-farm water temperature, nitrate and nitrite values. Generally the observed values were within the optimal range that has been recommended for normal tilapia growth.

The results for growth performance and survival rate of the three tilapia species (*O. ruvumae, O. hornorum and O. niloticus*) for both on-farm and on-station experiments are show in Table 7. Growth performance is indicated by final weight, weight gain, growth rate, final length and final width of the fish. For the on-farm experiment the mean final weight, weight gain, growth rate, final length, increase in length and final width differed significantly (P<0.001) among the species. Nile tilapia (*O. niloticus*) gained more weight (61.3 g) than *O. hornorum* (35.3 g) *and O. ruvumae* (13.8 g). The final weight, length and width of *O. niloticus* exceeded that of *O. hornorum* by 26.2 g, 2.4 cm and 0.7 cm, respectively. On the other hand the mean final weight, length and width of *O. hornorum* were higher than those of *O. ruvumae* by 23.6 g, 2.7 cm and 1 cm, respectively. For the on-station experiment the analysis of variance showed that there was no significant (P>0.05) difference between *O. niloticus* and *O. hornorum*, but the two species differed significantly (P<0.01) from *O. ruvumae*. As for the on-farm experiment, the *O. niloticus* had the highest final weight (26.7 g), weight gain (24.2 g), growth rate (0.3 g/d), final length (11.5 cm) and final width (3.3 cm) and it was followed by *O. hornorum.* The *O. ruvumae* showed poor performance in all parameters.

When the results for on-farm and on-station experiments were compared, the analysis of variance showed that location influenced significantly (P<0.05) the growth performance of the fish throughout the study period. The growth performances of *O. niloticus* and *O. hornorum* were significantly (P<0.05) higher in the on-farm experiment than in the on-station experiment, but that of *O. ruvumae* were not significantly different between the two locations. The values observed in the on-farm experiment for final weight, weight gain, growth rate, final length and width exceeded the values observed in the on-station experiment by 40.9 g, 37.1 g, 0.4 g/d, 3.6 cm and 1.2 cm, respectively for *O. niloticus. For O .hornorum* the on-farm values were higher by 14.8 g, 13.5 g, 0.16 g/d, 0.6 cm and 0.4 cm than the on-station values for final weight, weight gain, growth rate, final length and width, respectively.

Table 7 also shows the results for survival rate. Survival of the fish was significantly influenced by the species and location. In both experiments the highest survival rate (85.6% (on-farm) and 100% (on-station)) was observed on *O. niloticus*, followed by *O. ruvumae*. *Oreochromis urolepis hornorum* had the lowest survival rate (63.5 – 66.7%) in both experiments. For all species, fish reared at the on-station showed the higher survival rate (66.7 – 100%) compared to those reared in farmers' ponds (63.5 – 85.6%).

A total of three fish from each species were analyzed for chemical composition of their bodies. The results for chemical composition of the fish are indicated in Table 8 below. For the on-farm experiment the species did not differ significantly in dry matter and ash contents, but differed significantly in crude protein and fat contents. *Oreochromis hornorum* had the highest CP content (58.09%) and ether extract (30.12%) while *O. niloticus* had the lowest values (52.23% CP and 16.83% EE). For the on-station experiment the DM, CP and ash contents of the species were not significantly different. The CP content was slightly higher in *O. niloticus* (62.86%) and lower in *O. ruvumae* (56.3%). On fat content, *O. hornorum* had significantly higher EE (36.75%) compared to the other species which had EE contents between 16.92 (*O. niloticus*) and 18.77% (*O. ruvumae*). *Oreochromis niloticus* showed significant (P<0.05) difference in CP contents between the fish reared on-farm (52.23%) and on-station (62.89).

The results for economic analysis (Table 4) revealed that the highest total revenues per ha from sales of fish was observed on farmers who were raising *O. niloticus* (TZS 6,086,250.00 ≈ US$ 3518.06) while the farmers who culture *O. ruvumae* had the lowest revenue (TZS 1,584,000.00 ≈ US$ 915.61). Likewise the

highest variable costs per ha (2,577,280.00 ≈ US$ 1460.86) was found on fish ponds of the farmers who cultured *O. niloticus* while those who cultured *O. ruvumae* had the lowest variable cost (2,359,080.00 ≈ US$ 1363.63). The farmers who cultured *O. niloticus* had the highest profit per ha (3,558,970.00 ≈ US$ 2,057.21), followed by those who cultured *O. hornorum* (1,262,320.00 ≈ US$ 729.67). The culture of *O. ruvumae* resulted into a loss of TZS 775,080.00 (≈ US$ 448.02) per ha.

CONCLUSIONS

1. The Nile tilapia (*Oreochromis niloticus)* is superior to Jipe tilapia (*Oreochromis jipe*), Wami tilapia (*Oreochromis urolepis hornorum)* and Ruvuma tilapia *(Oreochromis ruvumae)* in terms of growth performance and survival rate.
2. The Wami tilapia (*Oreochromis urolepis hornorum)* is better than the other species (*Oreochromis jipe* and *Oreochromis ruvumae)* and can be cultured in ponds and give performace like that of Nile tilapia.
3. The culture of Nile tilapia (*O.niloticus*) in small-scale fish ponds is more profitable than the culture of Wami tilapia (*O. hornorum*) and Ruvuma tilapia (*O. ruvumae*).
4. Instead of looking for other tilapia species to replace the Nile tilapia, other means of improving the performance of the Nile tilapia should be sought, including selective breeding and better feeding.

REFERENCES

Hussain M.G., Kohinoor A.H.M., Islam M.S., Mahata S.C., Ali M.Z., Tanu M.B., Hossain M.A. and Mazid M.A. 2000. Genetic evaluation of GIFT and existing strains of Nile Tilapia, *Oreochromis niloticus* L.,under on-station and on-farm conditions in Bangladesh. *Asian Fisheries Science* 13: 117-126

Kaliba A.R., Osewe K.O., Senkondo E.M., Mnembuka B.V. and Quagrainie K.K. 2006. Economic Analysis of Nile Tilapia (Oreochromis niloticus) Production in Tanzania. *Journal of the World Aquaculture Society* 37 (4): 464 – 473.

Neves P. R., Ribeiro R. P., Vargas L., Natali M.R.M., Maehana K. R. and Marengoni N.G. 2008. Evaluation of the Performance of Two Strains of Nile Tilapia (*Oreochromis Niloticus*) In Mixed Raising Systems. *Brazilian Archives of Biology and Technology* 51 (3): 531 – 538

Rice, M.A., A. J. Mmochi, L. Zuberi and R.M. Savoie. 2006. Aquaculture in Tanzania. *World Aquaculture* 37(4):50-57

SAS (1998). Statistical Analysis System, SAS/STAT user's guide. Statistical Analysis Institute, Inc., Cary, NC, USA.

URT. 1997. National Fisheries sector policy and strategy statement. Ministry of Natural resources and Tourism, Dar es Salaam, Tanzania.

Table 1: Water quality parameters in the experimental fish ponds

Variable	**On – Farm Mean values**	**On–station – Mean values**
Temperature ^{0}C	25.1 ± 1.3	25.2 ± 2.0
DO	5.14 ± 1.4	5.4 ± 1.0
pH	7.4 ± 0.7	6.7 ± 0.2
Transparency	34.4 ± 8.2	42.2 ± 7.6
Nitrate	0-0.5	0-0.5
Nitrite	0-10	0-10

Table 2: Comparison of growth performance (mean ± se) of different tilapia species reared on-station and on-farm

Variable	**On–farm Species**			**On–station Species**			
	Oreochromis urolepis hornorum	*Oreochromis niloticus*	*Oreochromis ruvumae*	*Oreochromis urolepis hornorum*	*Oreochromis niloticus*	*Oreochromis ruvumae*	*Oreochromis jipe*
FnWt (g)	41.2±2.4^{b}	67.6±2.4^{a}	17.6±2.4^{d}	26.4±3.8^{c}	26.7±3.1^{c}	23.4±3.2^{cd}	16.3±2.0^{d}
Wt gain (g)	35.3±2.5^{b}	61.3±2.5^{a}	13.8±2.5^{d}	21.8±4.0^{c}	24.2±3.2^{c}	17.4±3.3^{cd}	12.3±2.0^{d}
GR (g/d)	0.4±0.03^{b}	0.7±0.03^{a}	0.15±0.03^{d}	0.24±0.04^{c}	0.3±0.04^{c}	0.2±0.04^{cd}	0.14±0.02^{d}
SGR	2.04±0.14^{a}	2.2±0.14^{a}	1.61±0.14^{b}	2.0±0.22^{a}	2.4±0.2^{a}	1.23±0.2^{b}	1.5±0.1^{b}
FnL (cm)	12.7±0.2^{b}	15.1±0.2^{a}	10.0±0.2^{d}	12.1±0.4^{b}	11.5±0.3^{bc}	11.0±0.3^{c}	10.15±0.3^{c}
FnWD (cm)	3.8±0.1^{b}	4.5±0.1^{a}	2.8±0.1^{d}	3.4±0.1^{c}	3.3±0.1^{c}	3.0±0.1^{d}	2.7±0.1^{d}
Length gain (cm)	6.0±0.3^{b}	8.5±0.3^{a}	4.1±0.3^{c}	6.0±0.5^{b}	6.3±0.4^{b}	4.0±0.5^{c}	
Survival rate (%)	63.5	85.6	78.0	66.7	100	95.8	95.8

Table 3: Comparison of chemical composition (mean ± sd) of different tilapia species reared on-station and on-farm

Location	Species	DM (%)	CP (%)	EE (%)	Ash (%)
On - farm	*O.niloticus*	27.99±3.13[a]	52.23±2.7[b]	16.83±1.64[b]	13.27±1.37[a]
On – farm	*O.hornorum*	25.59±1.95[a]	58.09±1.2[a]	30.12±10.86[a]	14.46±1.53[a]
On - farm	*O.ruvumae*	30.74±0.48[a]	53.15±0.66[b]	17.9±2.83[b]	13.56±0.28[a]
On - station	*O.niloticus*	33.83±8.49[a]	62.86±3.91[a]	16.92±2.8[b]	14.08±0.30[a]
On – station	*O.hornorum*	27.50±1.92[a]	55.86±5.88[a]	39.75±4.45[a]	15.80±0.28[a]
On – station	*O. ruvumae*	29.35±3.4[a]	56.30±5.23[a]	18.77±3.92[b]	14.40±2.12[a]
On - station	*O.jipe*	25.76±1.17[a]	61.46±1.09[a]	17.46±1.15[b]	14.92±0.57[a]

Table 4: Gross margin analysis of the different tilapia species cultured in ponds of small-scale farmers

Parameter	**Species**		
	O.niloticus	*O.hornorum*	*O.ruvumae*
Fingerlings costs/ha	1,600,000.00	1,600,000.00	1,600,000.00
Feed costs/ha	237,280.00	155,680.00	69,080.00
Labour/ha	540,000.00	540,000.00	540,000.00
Transport	150,000.00	150,000.00	150,000.00
Total variable costs/ha	2,527,280.00	2,445,680.00	2,359,080.00
Fish yield, kg/ha	1352.50	824.00	352.00
Fish price/kg	4500	4500	4500
Total revenue/ha from sales of fish	6,086,250.00	3,708,000.00	1,584,000.00
Profit/ha	3,558,970.00	1,262,320.00	-775,080.00

Note: The costs and revenue are computed in Tanzanian shillings (1730 TZS = 1 US$)

Training Program in Propagation and Hatchery Management of Tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*) in Ghana

Quality Seedstock Development/Activity/09QSD05PU

Stephen Amisah
Kwame Nkrumah University of Science & Technology
Kumasi, Ghana

Kwamena Quagrainie
Purdue University
West Lafayette, Indiana, USA

Joseph Padi and Felix Attipoe
Aquaculture Research Development Centre – Water Research Institute
Akosombo, Ghana

ABSTRACT

Increasing government support for aquaculture development in Ghana has the potential to create new jobs and improve food security among poor households. Unfortunately, technical know-how and skills in fingerling production is fairly restricted and most fish farmers lack the basic skills required for a successful fish production regime. Training programmes in fish propagation and hatchery management of Tilapia (*Oreochromis niloticus)* and Catfish *Clarias gariepinus* were conducted in Ghana at two separate locations in the Eastern and Ashanti regions. The training targeted small to medium scale fish farmers and potential fish farmers to provide them with technical knowledge and skills to enhance sustainable production of Tilapia and catfish fingerlings from hatchery stage to maturity. Over 60 fish farmers were trained in hatchery management and propagation of tilapia and catfish. It is anticipated that the skills acquired would enhance capacity of farmers and result in sustainable production of tilapia and catfish fingerlings to cope with the rising demand for fingerlings for commercial fish farming.

INTRODUCTION

Whilst fish consumption per capita in Ghana is estimated to be as high as 20kg compared to the global average of 13kg per capita, the fish farming sector is confronted with several problems. In the aquaculture sector, technical problems associated with fish production, particularly the lack of quality fingerling supply has limited the supply of market size fish to meet increasing demand. Some of the major setbacks to the production of fish to meet the increasing fish demand in Ghana are limited hatchery facilities, lack of proper management in hatcheries, and lack of quality fingerlings. Knowledge on the propagation and hatchery management of early stages of the Nile Tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus*) remains considerably restricted. The shortfalls of quality fingerlings seriously affect small- and medium-scale commercial fish farms, with a considerable number of farmers continuing to rely heavily on fingerlings harvested from the wild.

On the strength of these setbacks, a joint action by the Department of Fisheries and Watershed Management of Kwame Nkrumah University of Science and Technology, Ghana, the Ghana Fisheries Commission and the Aquaculture Research Development Centre - Water Research Institute (ARDEC-WRI), Ghana as well as Purdue University, USA, was undertaken to train fish farmers and potential fish farmers alike to acquire knowledge and practical skills in various aspects of fish hatchery, including

hatchery location and design, construction, operation and management; and fry and fingerlings production for the benefit of the small- and medium-scale sector of the aquaculture industry in Ghana. The joint action is in consonance with the pursuit of the national agenda to ensure food security and poverty reduction through fish farming to meet domestic fish requirements. There is, therefore, a need to focus on hatchery production and management, and fingerling production.

Training Location

To facilitate a considerable outreach and coverage for farmers in the country, the training sessions were conducted at two locations in Ghana: Aquaculture Research Development Centre (ARDEC-WRI) at Akosombo in the eastern region of Ghana to cater for those (but not exclusively) located in the Eastern, Volta, and Greater Accra regions. The other training session was held at the Fisheries Commission's Pilot Aquaculture Centre at Tano-Odumasi near Kumasi to cater for the training needs of the Ashanti, Brong Ahafo and the adjoining regions.

METHODOLOGY

A 2-day practical hands-on approach was adopted for the workshop training of farmers at both locations. The program followed both the lecture format with classroom presentations / lecture and a practical hands-on session where workshop participants handled fish for various purposes. Participants handled fish to examine and differentiate the sexes, identify mouth brooders incubating their eggs in the mouth, strip fish for eggs, and for pituitary injection.

Topics covered in the training program included the following:

- Spawning: natural and artificial reproduction strategies of fish.
- Managing eggs in mouth brooding tilapia
- Hatchery management from egg, larval fish, fry, juvenile, and consequently grow-out into market size adults.
- Management of fingerling production stages.
- Developing strategies for induced spawning in the African catfish
- Sex reversal techniques for Tilapia
- Water quality management and threats to fish life stages
- Packaging and transporting fingerlings

OUTPUT

The program trained over 60 fish farmers, 3 extension agents from the Fisheries Commission, several prospective fish farmers, and at least 2 research scientists. Each received practical-hands on approach to managing and handling fish from their early stages.

Each participant was equipped with knowledge and skills in:

- Identifying stages of the fish life cycle and identifying the progression from egg, larval fish, fry, juvenile, to adult and
- Describing natural and artificial reproductive strategies of fish
- Contrasting the reproductive strategies of tilapia and catfish.
- Hands-on learning in fish early life cycle development management from spawning through the fingerling production stages.

The training brought together collaboration of fish farmers, the Fisheries Commission, the Aquaculture Research Development Centre of the Water Research Institute, Council for Scientific and Industrial Research, Purdue University and an aquaculture expert from Kenya.

Brochures were produced that described the life cycle and reproductive strategies of Tilapia (*Oreochromis niloticus*). *Clarias gariepinus* is produced via induced spawning (hypophysisation) on the farm and induced spawning by adult fish and sex reversal techniques were taught during the training.

DISCUSSION

The training was found to be very timely and beneficial at a time when the government of Ghana is encouraging people to go into fish farming through the formation of workers brigade, cooperatives, farmer organizations, etc. It is hoped that the training will have a ripple effect as trained farmers will help train others or share new information from the training. It is expected that the knowledge acquired by the farmers will enhance their capacity to produce and manage their farms and hatcheries to improve quality fingerling production and ultimately aquaculture production to meet the deficit in fish demand.

CONCLUSION

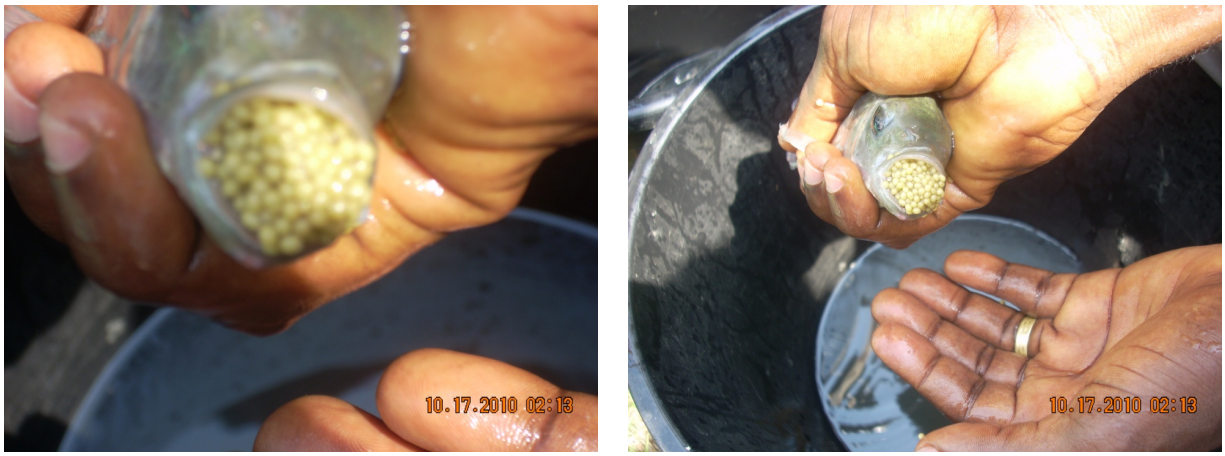
The training has been successfully accomplished and farmers who participated have suggested that the training be repeated periodically for upcoming farmers and for the benefit of areas that could not be reached. On their part they would teach other farmers the techniques they learnt to sustain the effort.

ACKNOWLEDGEMENTS

We wish to acknowledge Dr. Seth Agyarkwah of the Water Research Institute (CSIR), Ghana, Mr. Mantey Mensah, former Director of the Directorate of Fisheries, Ghana, Mr. Poku Gyinaye of BIAV Fish farms, Ghana and Mr. James Mugo of Mwea Fish Farm, Kenya for their diverse contributions and inputs in the running of the training program.

APPENDICES

Photos courtesy of Steve Amisah



A workshop participant handling Nile tilapia to show incubating eggs in the mouth

A workshop participant handling African catfish to differentiating between male and female; male on the left and female on the right

Workshop participants stripping catfish for eggs and another performing a pituitary injection.

Co-Management and Bivalve Sanitation for Black Cockles (*Andara* spp.) in Nicaragua

Human Health Impacts of Aquaculture/Experiment/09HHI01UH

Nelvia Hernández, Erick Sandoval and Juan Ramón Bravo
Central American University
Managua, Nicaragua

Maria Haws
University of Hawai'i at Hilo
Hilo, Hawai'i, USA

ABSTRACT

Black cockles (*Anadara tuberculosa* and *A. similis*) are an economic mainstay of many poor coastal communities in Latin America with over 2000 Nicaraguans directly benefitting from the harvest alone. Over-harvest and mangrove removal have reduced populations and the current regulatory system has been inadequate to fully address the situation. Beginning in 2006 as part of an integrated coastal management program (SUCCESS/USAID), and continuing under the auspices of AquaFish CRSP, several efforts began with the communities of the Aserradores Estuary to improve shellfish sanitation issues, increase direct revenues to cockle collectors and test whether voluntary, community-managed no-take zones would prove to be a feasible alternative management methods for cockles. Four no-take zones (25.56 ha total) were chosen, managed and monitored with direct participation by cockle collectors. After five years of monitoring, results indicate that *A. tuberculosa* cockle populations increased significantly both inside the no-take zones and in adjacent áreas. Increases were also observed for nearly all size classes for *A. tuberculosa*, but particularly for the smaller size classes, suggesting that the no-take zones resulted in increased recruitment. There was no significant change in *A. similis* populations. Following the success of this model, similar efforts are being pursued with other coastal communities in coordination with government agencies.

INTRODUCTION

Black cockles (*Anadara tuberculosa* and *A. similis*) are the most commonly harvested bivalves in Nicaragua, and are also a common fisheries target along most of the Pacific Coast of Latin America. In general, bivalve fisheries are generally overlooked in terms of fisheries management, with the result that in Latin America, most bivalve population are under considerable stress. This is also linked to issues with mangrove management, the habitat favored by *Anadara* spp. Lack of fully effective management methods and over-exploitation of cockle resources is considered to be an ubiquitous issue throughout Latin America (Mora and Moreno 2007, 2008; Silva and Bonilla 2001; Campos et al. 1990).

Cockle management began in Nicaragua in 1985 with the passing of regulations intended to protect cockle stocks. These regulations established an annual closed season from April 21 to June 15. In 2008, additional regulations were approved that addressed cockle management issues in protected areas, many of which are inhabited by, and still fished by fishers. These regulations have not been effective in maintaining cockle populations, in part because the closed season is not fully respected by fishers. Enforcement is difficult in the remote coastal areas covered by dense mangroves. It also does not necessarily protect cockles during their peak reproductive season. Moreover, coastal inhabitants are driven by the need to collect cockles for daily subsistence and modest income (Cheves 2011); many cockle collectors have few alternatives other than cutting mangroves for firewood.

In an effort to find more effective means of maintaining cockle populations while not adversely affecting the fishers, in 2006 CIDEA began working with the Aserradores community to test alternative management methods, improve shellfish sanitation and increase benefits to collectors with support from the USAID SUCCESS program. Additional CRSP support in 2009 allowed for continuation and expansion of these efforts until 2012. The combined work over the last six years included:

- Water quality testing to determine which areas of the estuary had sufficiently good water quality to allow for harvesting of cockles that are safe to eat (SUCCESS/USAID and CRSP 07IND0UH);
- Testing of field and laboratory depuration methods and rates (CRSP 07IND0UH);
- Development of a solar-powered, community operated depuration center (European Union); and
- Establishment and monitoring of no-take zones to increase cockle populations (CRSP 09HHI01UH).

This report addresses activity #4, which was the focus of the recent investigation (09HHI01UH).

Study site

The Aserradores Estuary is located on the Pacific coast of Nicaragua in the Department of Chinandega, approximately 169 km from the capital of Managua (Figure 1). There are approximately 450 inhabitants in the villages living in 110 houses. The villages are relatively new settlements, being initially populated from neighboring areas by fishers looking for better disembarkation sites. Seventy-four percent of the residents fish, 24% harvest cockles and 1% cut mangroves for firewood (CIDEA 2007). Fishing volume and average fish sizes have declined, and the patterns of good fishing days have also changed. Previously, the fishers could count on at least 15 days of good fishing monthly, now they usually only have good catchs for 2-3 days per month (Reyes 2011). Some tourism is now growing in the area, but the decline in fishing has put increased strains on the cockle fishery.

The Aserradores Estuary has 3,976 ha of mangroves, the majority of which is red mangrove (CATIE 2001). The extensive mangrove forest is used for cockle collection and cutting for firewood.

Figure 1. Location of the community of Aserradores in the Department of Chinandega, Nicaragua.

Asseradores was chosen as original test site for this work in 2006 due to the high level of participation in the cockle fishery by its inhabitants. It was also considered a model site due to the generally poor economic status of the community and the multiple stressors on the community, as well as the surrounding habitats. This village exemplifies dozens of other small, coastal villages which depend on natural resources and which are socially marginalized.

The stakeholders who harvest cockles consist of 78 families in two small villages within the area. Ninety-four percent of these villagers depend largely upon cockle harvest. Complete data does not exist

for the entire area, but for one of the smaller villages (Teodoro King), there are 311 people, of which 157 are women. The population, like the rest of Nicaragua, is relatively young, with 64.3% of the population being 20 years of age or younger. Women-headed households are common. Only 0.3% of the population is over 65 years of age

MATERIALS AND METHODS

The approach used to develop a co-management system for cockle was based on principles for community participation in coastal management initiatives articulated in Olsen and Ochoa (2004). Work with the Aserradores community began in 2006 with a series of community visits, workshops and general awareness-raising activities. Part of these activities were exercises with the community to chose the estuary areas where the voluntary no-take management zones would be located. At the same time, CIDEA initiated other management activities with the communities such as testing depuration of the cockles to improve human health, and evaluate the possibility of certification to improve the price paid to the collectors.

Initially three no-take zones were selected with the communities, and three years later, another no-take area was added at the suggestion of the community. Currently the total area under management as one of the no-take zones is 26.56 ha (Table 1).

Table 1. Description of no-take zones.

No	Name	Size (ha)	Date declared as a no-take zone
1	Los Tonos	4.22	Sept. 6, 2006
2	Río Viejo	5.95	Sept. 6, 2006
3	Castepe	10.39	Sept. 6, 2006
4	La Chanchera	6.00	April 19, 2009

In choosing the no-take zones with the community, several criteria were used: 1) the sites would be distributed such that no segment of the cockle collecting population was inequitably affected by the prohibition on harvesting; 2) the areas should be easily marked and recognized; 3) historically cockles had been present and harvested there; and 4) were sites not subject to wide flucuations in salinity.

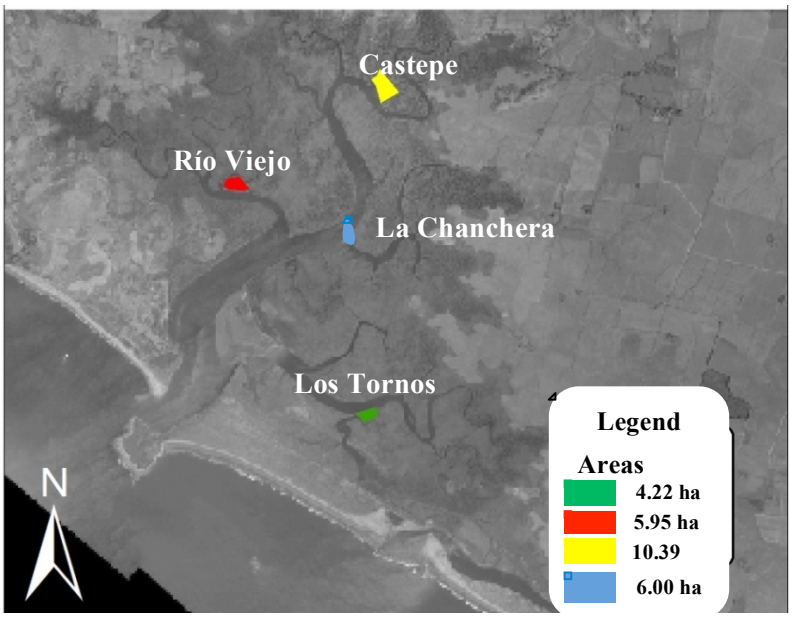


Figure 2. Location of the no-take zones within the Aserradores Estuary.

Each no-take zone was demarcated using painted plastic bottles which were hung in the surrounding mangrove trees above the high tide zone (Figure 3). The markers were periodically replaced. Meetings and visits within the cockle collecting villages were held to assure that everyone was familiar with the areas, the voluntary rules on collection and the potential benefits to the communities. General educational awareness presentations were also given.

Figure 3. Painted plastic bottles were used to mark the boundaries of the no-take zones.

To determine the cockle population density, a stratified sampling method was used. Fourteen 14 points within the estuary were selected for sampling. Six of these were located at points along the principal branch of the estuary (harvested areas) and two in or near each no-take zone. Of the latter two, one was within the boundaries of the no-take zone and one was located 100 meters from the no-take zone. Sampling in the latter was of interest to determine if a possible spill-over effect would result from the management of the no-take zones. Coordinates of the sampling sites are presented in Table 2 and a map of the sampling sites is shown in Figure 4. For each sampling event, three replicate plots (4 m^2 each) within each site were sampled. The participation of the cockle collectors, including children, was

essential for the sampling as cockles are difficult for non-experienced people to find, given their habit of living amongst mangrove roots. Even with this expert help, population numbers may be under-counted.

Table 2. Sampling sites to determine population densities.

No	Designation	Coordinates	
		X	Y
1	Point No.1	463656	1396888
2	Point No.2	464457	1396355
3	Point No.3	465273	1398249
4	Point No.4	465895	1397454
5	Point No.5	465072	1398514
6	Point No.6	465145	1398738
7	Los Tornos inside (within no-take zone)	465700	1394100
8	Los Tornos outside (adjacent to no-take zone)	465600	1394200
9	Río Viejo inside (within no-take zone)	463685	1397411
10	Río Viejo outside (adjacent to no-take zone)	463946	1397273
11	Castepe inside (within no-take zone)	465932	1398943
12	Castepe outside (adjacent to no-take zone)	465373	1399086
13	La chanchera inside (within no-take zone)	466243	1396571
14	La Chanchera outside (adjacent to no-take zone)	465845	1396458

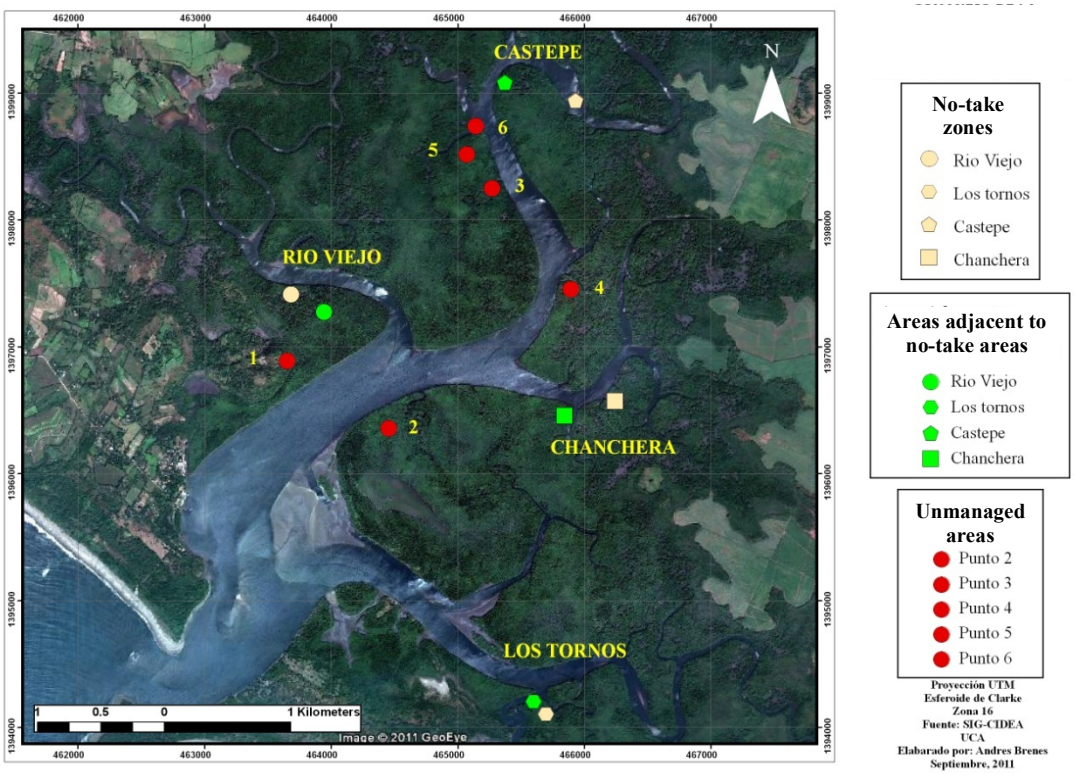


Figure 4. Map of the sampling sites within the Aserradores Estuary. The no-take zones are indicated, as well as the sampling sites that are adjacent to the no-take zones and sampling areas which were not under management, i.e. subject to uncontrolled cockle harvest.

Cockles (*Anadara tuberculosa* and *A. similis*) were then removed from each of the sample sites. Three replicate samplings were taken at each of the 14 sample sites and each sample comprised an area of 4 m^2. Between 2006 and 2011, eight sampling periods occurred. These represented months 0 (initiation of the trial), 6, 12, 23, 30, 49, 55 and 59 months. Data collected included the number of cockles at each site, and length (DVM) and weight. Data was analyzed using SPSS software for ANOVA, box plots and frequency histograms.

RESULTS

Population Densities

At the establishment of the no-take zones, minimum and maximum densities for both species were 1.42 ± 0.45 and 5.17 ± 0.45 cockles/m^2 , respectively, and the mean was 3.07 ± 0.45 cockles/m^2. For *A. tuberculosa*, the minimum density was 1.33 ± 0.37 cockles/m^2 and the maximum was 4.42 ± 0.37 cockles/m^2. For *A. similis*, the minimum density was 0.08 ± 0.08 cockles/m^2 and the maximum was 0.75 ± 0.08 cockles/m^2 (Table 3).

Table 3. Cockle densities at the beginning of the study. Minima and maxima are the average for each parameter among all the sites.

Population densities at the beginning of the study (cockles/m^2)	***A. tuberculosa* and *A. similis***	***Anadara tuberculosa***	***Anadara similis***
Initial minimum population density	1.42 ± 0.45	1.33 ± 0.37	0.08 ± 0.08
Initial maximum population density	5.17 ± 0.45	4.42 ± 0.37	0.75 ± 0.08
Initial mean population density	3.07 ± 0.45	2.71 ± 0.37	0.37 ± 0.08

Fifty-nine months after establishment of the no-take zones, the combined average densities for the two species had increased from 3.07 ± 0.45 to 8.02 ±1.08 cockles/m^2, representing an increase of 4.95 cockles/m^2. The minimum and maximum densities were 1.92 ± 1.08 cockles/m^2 and 19.75 ± 1.08 cockles/m^2 for both species. The final mean densities for the two species was 7.62 ± 0.97 cockles/m^2 (*A. tuberculosa*) and 0.40 ± 0.12 cockles/m^2 (*A. similis*) (Table 4).

Table 4. Cockle densities at the end of the study (after 59 months of management). Minima and maxima are the average for each parameter among all the sites.

Population densities at 59 months after initiation of the study (cockles/m2)	***A. tuberculosa* and *A. similis***	***Anadara tuberculosa***	***Anadara similis***
Final minimum population density	1.92±1.08	1.83±0.97	0.00±0.12
Final maximum population density	19.75±1.08	19.08±0.97	1.25±0.12
Final mean population density	8.02 ± 1.08	7.62±0.97	0.40±0.12

ANOVA analysis show that population increases for *A. tuberculosa* were significantly higher, but changes for *A. similis* densities over the 59 months of management were not significantly different (Table 5).

Table 5. ANOVA results for cockles densities at zero and 59 months.

		Sum of squares	df	Mean square	F	Sig.
Density (cockles/m^2) *Anadara tuberculosa*	Between groups	258.195	5	51.639	4.296	.002
	Within groups	769.340	64	12.021		
	Total	1027.535	69			
Density (cockles/m^2) *Anadara similis*	Between groups	.393	5	.079	.470	.797
	Within groups	10.699	64	.167		
	Total	11.092	69			

Given that changes in population densities over the 59 months of management did not change significantly for A. similis, the following discussion presents the detailed results for A. tuberculosa only.

The box plot for A. tuberculosa densities at all sampling sites shows some flucuations in the average density over the years between 2006 and 2011. The overall trend is an increase in densities. The fourth no-take area, "La Chanchera", which was established in 2009, always had population densities higher than at the other sampling points (Figure 5).

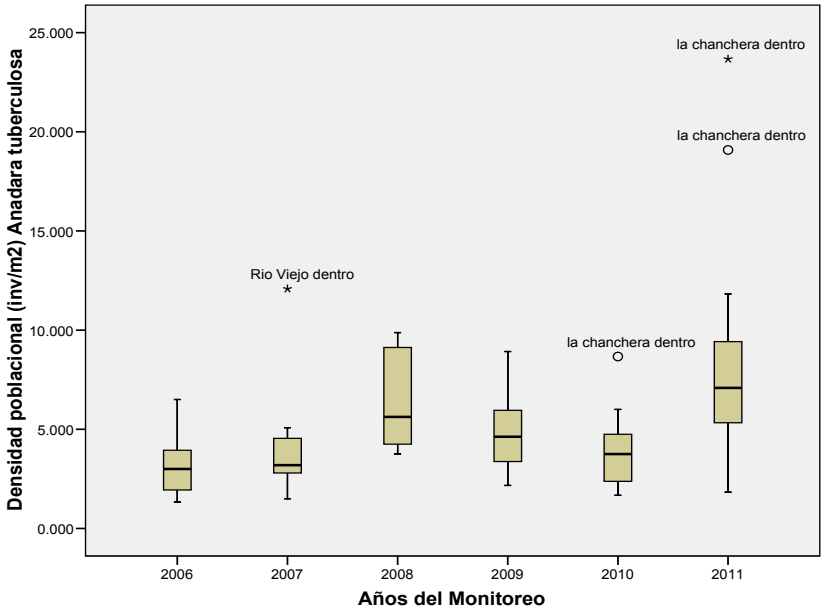


Figure 5. Box plot for *A. tuberculosa* densities combined for all sampling points.

The densities for the sampling points within the no-take zones and the adjacent areas, shows a pattern similar to the densities considered for all sampling points (Figure 6).

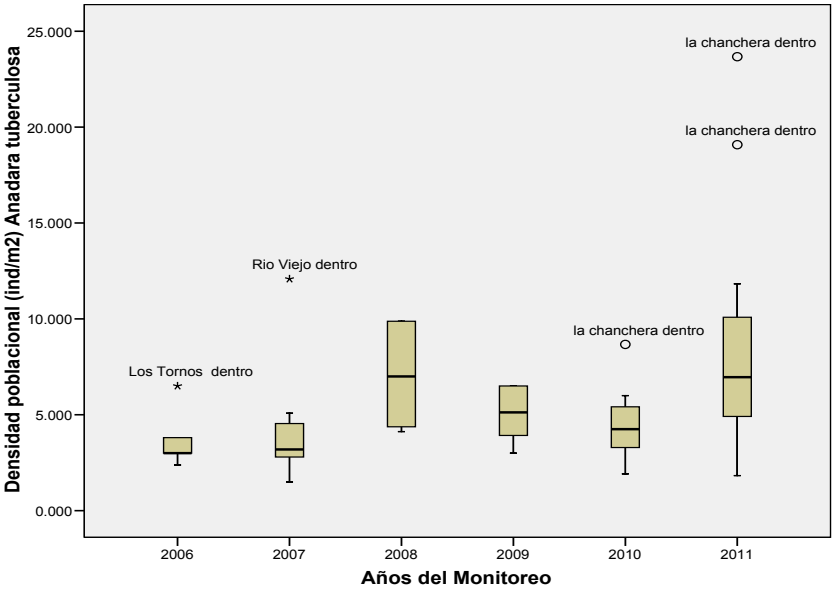


Figure 6. Population densities for *A. tuberculosa* for sampling stations within the no-take zones and the adjacent areas.

ANOVA results (Table 6) also indicate that the population densities for *A. tuberculosa* inside the no-take zones and the adjacent areas did not differ significantly. Figure 7 shows the densities for the sampling stations within the no-take zones, while Figure 8 presents the results from sampling points from areas where harvest was permitted.

Table 6. *A. tuberculosa* densities within the no-take zones and at adjacent sites.

	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between groups	164.151	5	32.830	2.056	.090
Within groups	670.728	42	15.970		
Total	834.878	47			

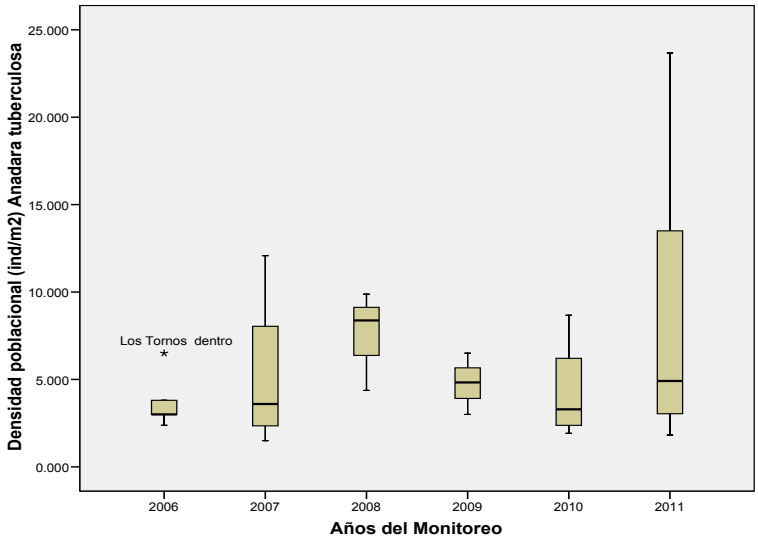


Figure 7. *A. tuberculosa* population densities in the no-take zones.

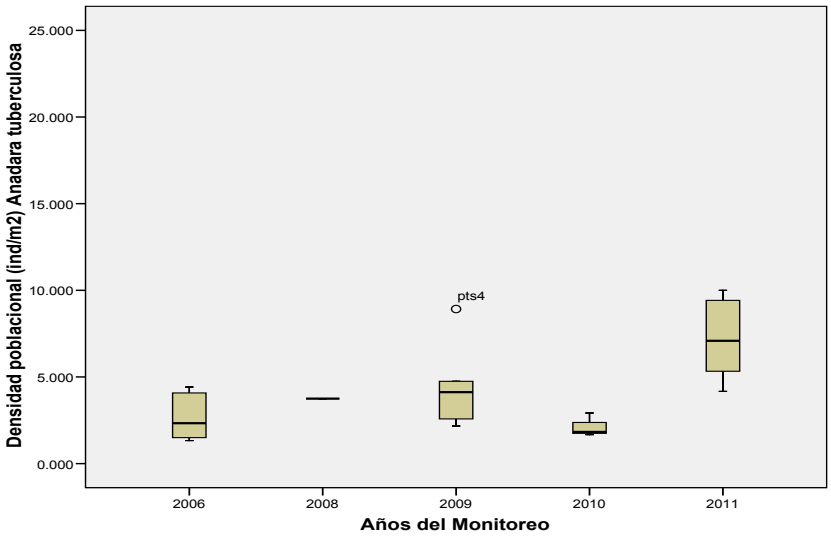


Figure 8. *A. tuberculosa* population densities in the areas where harvest was allowed.

At the end of 2011, the mean population density for all sites increased significantly (Figure 9, blue histogram). The highest individual counts encountered among the sites also increased from 11 to 22 cockles, although flucuations in maximum counts varied over time (Figure 9, green trend line).

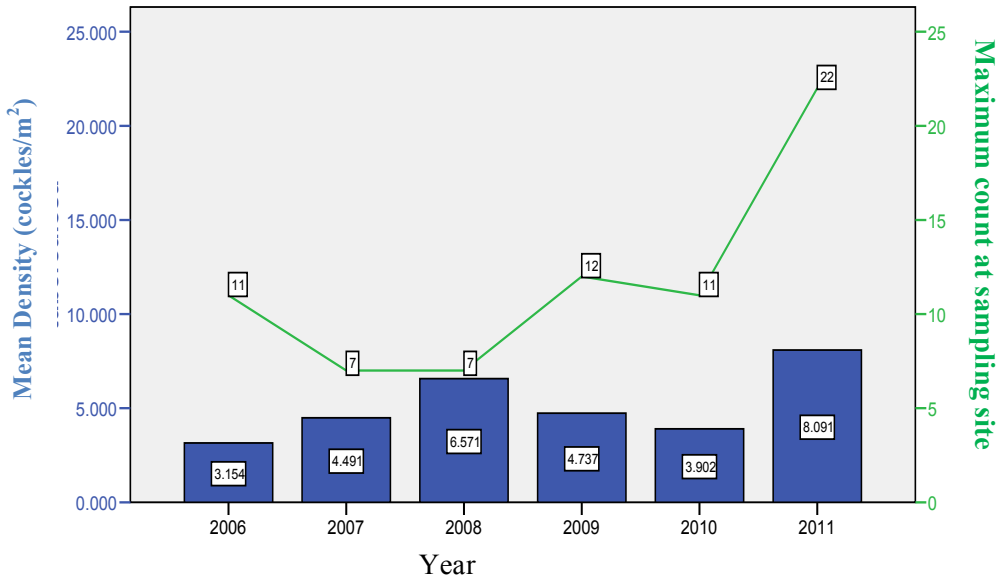


Figure 9. Mean densities for all sampling sites (in blue histogram) and maximum count at any sampling site during the sampling period (green line). In the case of years where two samplings were conducted, results are combined to represent the average for that year.

Population structure

According to the distribution histogram, there was an increase in all size classes, but particularly for the size classes between 5 and 50 mm for *A. tuberculosa* (Figure 10).

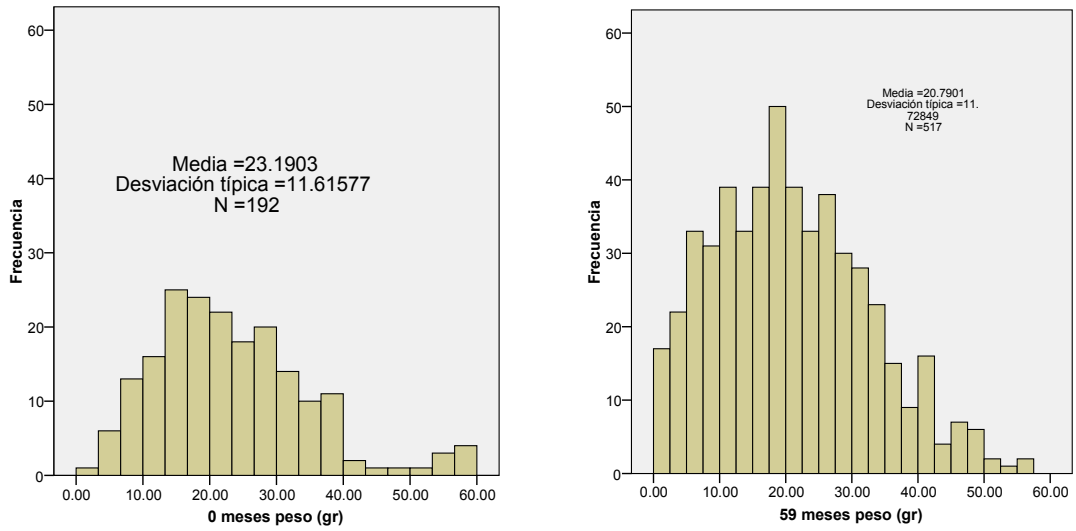


Figure 10. Size class distributions at months 0 (left) and 59 (right) for *A. tuberculosa*.

DISCUSSION AND CONCLUSION

Information on cockle population densities is not available for the Aserradores Estuary for periods prior to initiation of this research. Arrechavala and Estrada (2004) found population densities for cockles at six Nicaraguan estuaries ranged from 0.005 to 0.82 cockles/m^2 for *A.tuberculosa* and 0.002 to 0.6 m^2 for *A. similis*. Silva and Bonilla (2001) found that *A. tuberculosa* densities in Costa Rica were 0.8±0.1 to 1.2±0.1 cockles/m2. Densities in Ecuador were reported as 3.74±0.72 ind/m^2 (Mora and Moreno 2006).

Since cockle densities for both species increased from 3.07 ± 0.45 cockles/m^2 in 2006 to 8.02 ± 1.08 cockles/m^2 in 2011, representing an increase of 4.95 cockles/m^2 in 59 months, this suggests that the no-take zones were effective in protecting the cockle population and led to an increase in population densities. The final results also compare favorably to the cockle densities found in other heavy fished estuaries in Nicaragua, Costa Rica and Ecuador. For the species *A. tuberculosa*, increases in densities over the 59 month period led to mean final densities (19.75 cockles/m^2) that were also much higher than those reported elsewhere. While *A. similis* populations did not display a signficant change in population densities, the densities at least did not decrease, which may have been a possibility considering the intensity of the cockle fishing in the area. One issue with both the lack of significant increases of *A. similis* over the 59 month period, as well as the flucuations in densities between years may be sampling error. Cockle distribution tends to be patchy. They can also be difficult to find and although most of the cockle collectors participating in the monitoring tended to participate throughout the five years, there could have been some change in either the expertise or level of effort among the collectors.

The differences in population densities for *A. tuberculosa* and *A. similis* at the final stages of this study are not dissimilar to differences in populatio ratios for the two species encountered in other studies. In Ecuador the ratio of *A. tuberculosa* to *A. similis* was found to range from 3.5:1 to 4:1 (Mora and Moreno 2006). The two species also occupy slightly different habitat types within the estuary. *A. tuberculosa* tends to be found in clay-like soil under *Rhizophora mangle* roots at a depth between 5-30 cm while *A. similis* is found between 15 and 50 cm in soft substrates in subtidal areas, often in open areas not covered by mangrove (Mora 1990; Fisher et al. 1995; Borda and Cruz 2004). *A. similis* may therefore be more subject to harvest and thus may need additional no-take zones to fully protect this species.

The highest counts from any sampling site at each sampling period increased from 11 in 2006 to 22 in 2011. This information is particularly persuasive to cockle collectors since their livelihoods depends on being able to readily locate patches of cockles with high counts in order to improve the efficiency of collection, particularly since collection time is limited by the tides. The ability to locate and collect large numbers of cockles also affects the cost per unit for collection since collectors may rent canoes to access some areas. Collectors reported that even at the first sampling period (6 months), their perception was that more cockles, particularly small cockles, were found in and around the no-take zones.

Previous studies suggest that *Anadara* cockles may reproduce up to three times yearly (Garcia et al. 2008; Maldonaldo 2005). Reproductive size is believed to be between 23.2 and 26.2 2 mm (Ampie and Cruz 1988) with the maximum size of 81 mm being reached in five years (de Madrigal 1980; Campos et al 1990; Borda and Cruz 2004) for *A. tuberculosa*. The legal minimum size for the two species is 40 mm for *A. similis* and 50 mm for *A. tuberculosa*. Within the five year period of this study, at least two generations of cockles should have reached sexual maturity. The increases in the size classes under 50 mm may be reflecting increased recruitment and growth of the smaller size classes. The lack of change in size classes above 50 mm and the lack of very large cockles, may indicate the continuing intensity of fishing for larger, legal sizes.

An important component of this work was the collaboration with other institutions and communities in order to replicate this work if the establishment of no-take zones as a management method proved to be successful. UCA collaborated closely with INPESCA, FUNDAR, MARENA, UNAN-León to conduct the research and to raise awareness of cockle management issues and approaches in other coastal communities. This included several efforts not originally included in the planned work, but which will contribute signficiantly to improved management of bivalve sanitation and fisheries management. UCA is now developing a solar powered depuration center in the Aserradores community. Government agencies and other communities are also now evaluating the use of no-take zones as a legally acceptable management method. Additionally, the institutions involved in this work also collaborated to develop a strategic management and development plan (2012-2016) for the cockle sector. Results from this work were presented at numerous community workshops, and two regional workshops (Cental America and Mexico) supported by CRSP. The latter is described in the final technical report for CRSP investigation 09HHI02UH.

This work demonstrates that this co-management method based on no-take zones can be an effective alternative management method. The successful implementation of this approach is only effective when both the community and the regulatory agencies fully engage with, and participate in the efforts. Long term sponsor support is also key. Time is needed to lay the ground work to implement no-take zones, as well to detect changes in populations densities and structure that are not only scientifically valid, but are observable to the communities supporting the efforts and sacrificing some of their gathering areas. Continual efforts to keep the community informed as to the results is also important to maintain their interest and support.

LITERATURE CITED

Arrechavala, C. and Estrada, R. 2004. Evaluación ecológica y bromatológica de las especies *Anadara tuberculosa* y *Anadara similis* en los departamentos de León y Chinandega. Universidad Centroamericana. Tesis.

Ampie, C. and Cruz, A. 1988. Tamaño y madurez sexual de *Anadara tuberculosa* (Bivalvia:Arcidae) en Costa Rica.

CATIE. 2001. Estrategia para el Desarrollo y la conservación de la zona costera municipio El Viejo, Nicaragua/CATIE, Alcaldía Municipal El Viejo, Instituto de Desarrollo Rural, Ministerio de Ambiente y Recursos Naturales. Turrialba, CR. 98 p Serie técnica No 321.

Campos, J. Founier, M. Soto, R. (1990) Estimación de la población de *Anadara tuberculosa* en Sierpe – Terraba Costa Rica. Rev Biol. Trop. 38: 477-480

Borda, C.A. and R. Cruz. 2004. Crecimiento y tasas de mortalidad del bivalvo *Anadara tuberculosa* (SOWERBY, 1833) en el Pacífico Colombiano. Rev. Invest. Mar. 25(3):177-184.

Centro de Investigación de Ecosistemas Acuáticos (CIDEA). Universidad Centroamericana. 2007. Linea de base y trayectoria del cambio, comunidad de Aserradores. Municipio de El Viejo. Managua, Nicaragua. 40 pp.

Centro de Investigación de Ecosistemas Acuáticos (CIDEA). Universidad Centroamericana. 2005. Estudio de mercado de moluscos, ostras, mejillones y almejas en Centroamérica y del mercado interno de conchas negras en Nicaragua. Managua, Nicaragua.

Cheves, C. (15 de Abril de 2011). Las conchas negras tienen rostro de pobreza extrema. (results of interview conducted by Nelvia Hernandez.).

Mora, e. and J. Moreno. 2006. Estado de la pesquería del recurso conchas (*Anadara tuberculosa* y *A. similis*) en la Costa Ecuatoriana. Instituto Nacional de Pesca. Ecuador.

García, F., A. Haro, A. García, and C. Marcial, F. Villalejo and S. Rodríguez. 2008. Ciclo reproductivo de *Anadara tuberculosa* (Sowerby, 1833) (Arcidae) en Bahía Magdalena, México. Revista de Biología Marina y Oceanografía 43(1): 143-152.

Hernández, N., A. Saavedra and C. Hernández. 2011. Diagnóstico sobre el estado de aprovechamiento del recurso conchas negras (*Anadara tuberculosa* y *Anadara similis*) en la costa pacífica de Nicaragua. Instituto CIDEA, Universidad Centroamericana.

Hernández, C. 1992. Diagnosis del recurso conchas de la región II del Pacífico de Nicaragua UNAN. León Nicaragua, 7 pag. No publicado.

Mora, E. and J. Moreno. 2007. Abundancia y estructura poblacional de *Anadara tuberculosa y A. similis* en las principales áreas de extracción de la costa Ecuatoriana. Informe técnico INP.

Mora, E. and J. Moreno. J. 2008. Variación temporal de las densidades de *Anadara tuberculosa* y *A. similis* en los ecosistemas de manglar de la costa ecuatoriana. Informe Tecnico

Maldonado, J. 2005. Aspectos Biológicos y Pesqueros de la Concha Prieta (*Anadara tuberculosa*) en Puerto El Morro entre septiembre y diciembre de 2005. Instituto Nacional de Pesca Ecuador. Investigación de los Recursos Bioacuáticos y su Ambiente.

Olsen, S. and E. Ochoa. 2004. Marco Metodológico y conceptual para la Planeación e Implementación del Manejo de Ecosistemas Costeros. Centro de Recursos Costeros Universidad de Rhode Island y Fundación EcoCostas. Guayaquil.

Rendón, M., E. Suárez and M. Mejía. date unknown. Manejo sustentable y comercialización de concha prieta en cautiverio en Puerto el Morro (Provincia del Guayas), para su exportación hacia España. Facultad de Ciencias Humanísticas y Económicas. ESPOL.

Reyes, J. 2011. (20 septiembre 2011). Owner of a cockle consolidation center in Aserradores. Personal Communication. (Interview by Nelvia Hernandez).

Resolución No. 243-2009. Reglamento técnico RTCA 67.04.50:08. Centroamericano. Alimentos. Criterios microbiológicos para la inocuidad de alimentos.

Silva, M. and R. Bonilla. 2001. Abundancia y morfometría de *Anadra tuberculosa* y *A. similis* (Mollusca Bivalvia) en Manglar de Purruja, Golfo Dulce, Costa Rica. Centro de Investigación en Cinecias del Mar y Limnología (CIMAR), Universidad de Costa Rica. San José.

Villalobos, C. and A. Baez. 1983. Tasa de crecimiento y mortalidad de *Anadara tuberculosa* (Bivalvia: Arcidae) en dos sistemas de cultivo en el Pacífico de Costa Rica.

Ville, C. W. Walkerand F. Smith. 1990. Invertebrados superiores. En: Biología. México, Editorial McGraw- Hill.

Capacity Building in Aquaculture, Fisheries Management and Coastal Management for Coastal Women Workshop: "Opportunities for Coastal Women in Fisheries, Aquaculture and Coastal Management"

Human Health Impacts of Aquaculture/Activity/09HHI02UH

Maria Haws
University of Hawai'i at Hilo
Hilo, Hawai'i, USA

Eladio Gaxiola Camacho and Lorena Irma Camacho Lopez
Universidad Autónoma de Sinaloa
Culiacan, Sinaloa, Mexico

Nelvia Hernandez, Erik Sandoval, Carlos Rivas,
Juan Ramon Bravo and Eufresia Balladares
Central American University
Managua, Nicaragua

ABSTRACT

Two regional workshops were held in Nicaragua and Mexico for 127 participants from Nicaragua, Mexico, Honduras and El Salvador to exchange experiences and knowledge in the fields of aquaculture, fisheries management and coastal management. These workshops were designed to highlight the achievements of women's groups. A video was also made which was shown on national televisión

INTRODUCTION

Since 1996, a team of UAS researchers has been working in coastal communities to develop coastal management efforts which culminated in two officially adopted coastal management plans (Bahia Santa Maria and Boca de Camichin). The findings and results from the Mexico work were published as part of the ACRSP program (Haws, et. al. 2008) and integrated into peer-reviewed publications as part of the USAID SUCCESS program (Crawford et. al. 2010; Haws et. al. 2010). Since that time, the same team and a number of partners from the education, government and private sectors have continued with implementation efforts following the recommendations of the management plans. A similar effort has been in place in Nicaragua since 1998, which led to the official adoption of the management plan for the Estero Real (Royal Estuary), a watershed which drains approximately 30% of Nicaragua and parts of Honduras and is a major tributary for the trinational Gulf of Fonseca. In both nations, the primary target groups have been socially-disadvantaged groups including women, fishers, disabled and indigenous groups. Women comprise approximately 80% of the total stakeholders involved. The range of the CRSP work, including leveraged efforts and similar projects have included topics such as: fisheries co-management, development of national regulations for previously unregulated fisheries species, alternative livelihoods (aquaculture, eco-tourism, handicrafts, bakeries), large-scale water quality monitoring, aquaculture best management practices, seafood sanitation and quality and bivalve sanitation. Two major cross-cutting themes in this work are conservation and food security. Although efforts have been made to disseminate the results of these many years of work for both the research and the extension efforts, more work needs to be done in this area. Additionally, although women are active and involved at all levels, the participation of women can be further enhance and their individual and collective capacity improved. This work proposed to accomplish this through holding two regional workshops, one in Nicaragua and one in

Mexico, to provide a venue for exchanging lessons learned and practical methods. Women, including young girls, were the primary audience for this work. Attempts were made to involve participants who had not had the opportunity to become involved previously.

SPECIFIC OBJECTIVES INCLUDED:

- Hold two regional workshops for women and female children to provide an opportunity to exchange lessons learned and identify future needs
- Inclusion of young women to broaden the participation of women in the future
- Provide training with a focus on alternative livelihoods (including aquaculture) that are specifically targeted to users of threatened natural resources (e.g mangrove wood cutters, shrimp post-larvae fishers)
- Produce an outreach video highlighting the achievements of each nation's women in these areas. This will be shown on TV channels in both countries and distributed to institutions working with these topics.

METHODS

Nelvia Herndez, Eufresia Balladares, Lorena Irma Camacho Lopez and Eladio Gaxiola took the lead on coordinating the planning and organization of the two workshops. Ms. Camacho Lopez (Sociologist) attended the workshop in Nicaragua and presented the coverage on the Mexico efforts. She was thus familiarized with the Nicaraguan efforts and was able to present that latter at the Mexico workshop. The Nicaragua workshop was regional in nature, with participants from Nicaragua, Honduras and El Salvador. The Mexico workshop was also regional, with women and community groups from the States of Sinaloa, Nayarit, Sonora, Baja California Sur and Baja California Norte.

RESULTS

The Nicaragua workshop on July 26-27, 2011 and had 96 participants. Session topics included:

- Development of an integrated plan for sustainable extraction (Gulf of Fonseca/El Salvador project).
- Social Capital-a critical aspect of the success of oyster culture in Sinaloa Mexico (presented by Lorena Irma Camacho Lopez)
- Seed production and culture of mangrove molluscs
- Black cockle enclosure culture system
- Program for alternative cockle fisheries management and shellfish sanitation
- Visit to the voluntary no-take zones for black cockles in Asseradores Estuary
- Visit to the cockle culture and tourism project in Las Peñitas-Leon

The Mexico workshop took place in Guamúchil, Sinaloa, México on September 3 and 4, 2011. There 31 participants on the first day and 28 on the second day. Session topics included:

- Exchange of experiences from Baja California Sur, "Exportation of ornamental species"
- Exchange of experiences from Nayarit State, "Women's association of Boca de Camichin for the development of limited resources"
- Exchange of experiences from Sonora State, "The only social cooperative of women oyster farmers"
- Exchange of experiences from Baja California Sur, "Women shellfish growers"

- Exchange of experiences from Sinaloa State, "Women's crab picking cooperative-production of crab meat"
- Exchange of experiences from Sinaloa State, "Women's production of handicrafts based on natural resources"
- Exchange of experiences from Sinaloa State, "Playa Colorada Mariculture Cooperative"
- Experiences from Nicaragua, "Women cockle collectors from Nicaragua".
- Tour of the oyster culture farm of the Playa Colorada Mariculture Cooperative.

The workshops were video-taped and have been shown on Nicaraguan television. The participants expressed a high level of satisfaction with the workshops and the exchanges of information and experiences were valuable for all.

CONCLUSION

The workshop was very beneficial for increasing the level of communication and cooperation between the four institutions and the multiple community stakeholders participating in this work.

BENEFITS

One hundred twenty-seven participants from four countries increased their knowledge of approaches to sustainable fisheries and aquaculture.

ACKNOWLEDGEMENTS

The LSU, UHH, UAS and UCA teams are grateful to the Aquaculture and Fisheries CRSP which provided funding and support for this effort.

REFERENCES

Crawford, B.C., M.D. Herrera, N. Hernandez, C. Rivas-LeClair, N. Jiddawi, M. Semba, M.C. Haws. 2010. Small scale fisheries management: lessons from cockle harvesters in Nicaragua and Tanzania. Coastal Management Journal. 38:195-215.

Haws, M.C., B. Crawford, S.C. Ellis, N. Jiddawi, A. Mmochi, E. Gaxiola-Camacho, G. Rodriguez-Dominguez, G. Rodriguez, J. Francis, C. Rivas-LeClair, A. Saborio-Coze, N. Hernandez, E. Sandoval, K. Dabrowski, M.C. Portella and M. Jaroszewska. 2010. Aquaculture research and development as an entry-point and contributor to natural resources and coastal management. Coastal Management Journal: 38: 238-261.

Haws, M.C., E. Ochoa-Moreno and A.A. Rojas Umana. 2008. (eds.). Human Health and Aquaculture: three case studies of the relationships between human health, the environment and aquaculture in coastal communities of Sinaloa, Mexico. Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University. 157 pp.