Feed the Future Innovation Lab for Collaborative Research on Aquaculture & Fisheries (AquaFish Innovation Lab)

Technical Report: Investigations 2016-2018

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AquaFish Innovation Lab Management Office, Oregon State University
Strand Agriculture Hall ◆ Corvallis, Oregon USA

USAID FROM THE AMERICAN PEOPLE

Oregon State University

AQUAFISH INNOVATION LAB

AQUAFISH INNOVATION LAB TECHNICAL REPORTS: INVESTIGATIONS 2016-2018

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Disclaimers

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Cover photo

Research ponds in Cambodia, 2018. (Photo by Jenna Borberg).

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AquaFish Innovation Lab
Oregon State University
Corvallis, OR 97331

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INTRODUCTION

The Feed the Future Innovation Lab for Collaborative Research on Aquaculture & Fisheries (AquaFish Innvoation Lab), previously the AquaFish Collaborative Research Support Program, operated under a five-year extension from USAID that was awarded in 2013. USAID (May 2013, RFA) looked to the AquaFish Innovation Lab to "develop more comprehensive, sustainable, eco-logical and socially compatible, and economically viable aquaculture systems and innovative fisheries management systems in developing countries that contribute to poverty alleviation and food security."

The AquaFish Innovation Lab was managed to achieve maximum program impacts, particularly for small- and mid-scale fish farmers and fishers, in Host Countries and more broadly, by addressing the need for world-class research, capacity building, and information dissemination. Specifically, it strived to:

- Develop sustainable end-user level aquaculture and fisheries systems to increase productivity, enhance international trade opportunities, and contribute to responsible aquatic resource management;
- Enhance local capacity in aquaculture and aquatic resource management to ensure long-term program impacts at the community and national levels;
- Foster wide dissemination of research results and technologies to local stakeholders at all levels, including end-users, researchers, and government officials; and
- Increase Host Country capacity and productivity to contribute to national food security, income generation, and market access.

AQUAFISH RESEARCH FRAMEWORK

This AquaFish Innovation Lab Technical Report contains the final investigation reports for research and capacity building activites that were underway from 2016-2018. The overall research context for the work described in this report is poverty alleviation and food security improvement through sustainable aquaculture development and aquatic resources management. Research that built on existing work to generate new information and established an exit strategy so that research and impacts could extend beyond the life of the AquaFish Innovation Lab formed the core of AquaFish 2016-2018 work. Institutional strengthening, gender integration, outreach, and capacity building were also integrated into the research portfolio through activities such as training, formal education, workshops, extension, and conferences to support the scientific research being conducted.

As part of its extension in 2013, AquaFish continued and restructured five projects that were underway in Asia and Africa, with research periods divided into two, two-year Implementation Plans. The first of these Implementation Plans took place from 2013-2015, and successful lines of research were continued under the 2016-2018 Implementation Plan. For each Implementation Plan, US and Host Country Project PIs from the five existing research projects were invited to submit continuing proposals by responding to an AquaFish request for proposals (RFP). These proposals underwent an NSF-style external technical peer-review process along with a programmatic review involving the AquaFish Management Team. Two additional US-based desk studies focused on fisheries and aquaculture in Burma were also initiated during the second Implementation Plan.

The final investigation reports described herein represent the results of the lines of research that were submitted by the five projects in response to the 2015 RFP as well as the additional Burma studies, and approved by Oregon State University (OSU), the overall Lead Institution for the AquaFish Innovation Lab.

PROGRAM REGIONS

Projects were focused in Africa and Asia, and collectively involved 9 Host Countries, which included Bangladesh, Burma, Cambodia, Ghana, Kenya, Nepal, Tanzania, Uganda, and Vietnam. Proposed activities received USAID country-level concurrence prior to award. Projects aimed to build on previous USAID investment by strengthening linkages to host countries, improving capacity and infrastructure within countries, and extending supportive activities to nearby countries within the same region.

GLOBAL THEMES (GOALS)

The four global themes of the AquaFish Innovation Lab were cross-cutting and addressed several specific USAID policy documents and guidelines, including the Feed the Future Initiative and Research Strategy. Each project focused on one primary AquaFish theme, yet balanced all four themes in an integrated systems approach for producing positive development outcomes.

A. Improved Human Health and Nutrition, Food Quality, and Food Safety
B. Income Generation for Small-Scale Fish Farmers and Fishers
C. Environmental Management for Sustainable Aquatic Resources Use
D. Enhanced Trade Opportunities for Global Fishery Markets

AQUAFISH TOPIC AREAS

All projects were organized around ten specific areas of inquiry called Topic Areas that pertain to aquaculture and the nexus between aquaculture and fisheries. AquaFish took a systems approach that incorporated natural and human sciences by requiring that each AquaFish project integrate topic areas across the following two categories: (1) Integrated Production Systems, and (2) People, Livelihoods, and Ecosystem Interrelationships. Some of the following topic areas overlap and are interconnected.

Integrated Production Systems

- Production System Design & Best Management Alternatives (BMA)
- Sustainable Feed Technology (SFT)
- Climate Change Adaptation: Indigenous Species Development (IND)
- Quality Seedstock Development (QSD)

People, Livelihoods, and Ecosystem Interrelationships

- Human Nutrition and Human Health Impacts of Aquaculture (HHI)
- Food Safety, Postharvest, and Value-Added Product Development (FSV)
- Policy Development (PDV)
- Marketing, Economic Risk Assessment, and Trade (MER)
- Watershed and Integrated Coastal Zone Management (WIZ)
- Mitigating Negative Environmental Impacts (MNE)

TECHNICAL REPORT ORGANIZATION

This AquaFish Innovation Lab Technical Report contains the final reports for investigations described in the 2016-2018 Implementation Plan (published 2016) and the subsequent addendum; as well as reports for five extended investigations from the 2013-2015 Implementation Plan. The reports are organized by Topic Area.

Final investigation reports contained in this technical report have been published as submitted, with light copy editing and formatting. Please contact the authors of a given investigation report directly for questions about content.

TOPIC AREA

PRODUCTION SYSTEM DESIGN AND BEST MANAGEMENT ALTERNATIVES

❖

EXPERIMENTAL POND UNIT ASSESSMENT IN TANZANIA

Production System Design and Best Management Alternatives/Experiment/16BMA01PU

Sebastian W. Chenyambuga[1], Nazael A. Madalla[1], Renalda Munubi[1], Kwamena Quagrainie[2], and Emmanuel Frimpong[3]

[1]*Sokoine University of Agriculture, Morogoro, Tanzania.*
[2]*Purdue University, USA.*
[3]*Virginia Polytechnic Institute and State University, USA.*

ABSTRACT

An experiment was carried out to compare the effects of pond fertilization alone, concentrate feeding alone at 5% of fish biomass and a combination of fertilization and feeding at 2.5% of fish biomass on water quality parameters and growth performance and yield of Nile tilapia. The experiment was conducted using nine earthen ponds with average size of 188.67 m^2 at Tindiga Village, Kilosa District, Tanzania. The treatments were weekly fertilization alone with urea and Di-Ammonium Phosphate (DAP) at a rate of 3 g/m^2 and 2 g/m^2, respectively (T_1), concentrate feeding alone at 5% of fish biomass (T_2) and weekly fertilization with urea and DAP plus concentrate feeding at 2.5% of fish biomass (T_3). The concentrate diet contained 30% crude protein. The ponds were stocked with sex-reversed Nile tilapia at a stocking density of 3 fingerlings per m^2. A random sample of 30 fish per pond was measured for body weight and length every two weeks. Dawn pond water pH, dissolved oxygen (DO), conductivity, total dissolved solids, salinity and temperature were measured weekly while diel measurements were taken nine times in 24 hours at an interval of 3 hours (i.e. at 0600 h, 0900 h, 1200 h, 1500 h, 1800 h, 2100 h, 2400 h, 0300 h and 0600 h) at the beginning, mid and end of the experiment. The experiment took 166 days. A second experiment was conducted using nine concrete tanks, each with a surface area of 3.36 m^2. The treatments used in this experiment were the same as those used in the first experiment. The sex-reversed Nile tilapia were stocked at the same stocking rate (3 fingerlings/m^2) and fed the same feed and the same feeding levels and frequency used in the first experiment. The purpose of this experiment was to complement the pond experiment.

Results indicate that in both culture system (earthen ponds and concrete tanks) treatment had significant effect ($p \leq 0.001$) on water pH, DO, conductivity, TDS and salinity but not on temperature and turbidity. Ponds under fertilization alone (T_1) (pH = 8.23 ± 0.02, DO = 4.03 ± 0.11 mg/L) and fertilization plus feeding (T_3) (pH = 8.20 ± 0.02, DO = 4.43 ± 0.11 mg/L) had higher ($p \leq 0.001$) water pH than those under the treatment of concentrate feeding alone (T_2) (pH = 8.08 ± 0.02, DO = 3.88 ± 0.09 mg/L). Results for diel measurements show that DO, pH and temperature values were lowest at 0600 hours, started to increase at 0900 hours and reached the peak at 1500 hours and then started to decrease and reached the lowest values at 0300 hours. In both earthen ponds and concrete tanks, treatment had significant effects on final body weight, weight gain, growth rate, specific growth rate and feed conversion ratio (FCR), but did not significantly affect survival rate. Fish cultured in ponds under T_1 had significantly lower weight gain (65.4 ± 1.1 g), growth rate (0.4 g/day) and specific growth rate (2.5%) than those cultured under T_2 and T_3. The weight gain (193.8 ± 4.5 g), growth rate (1.2 g/day) and specific growth rate (3.3%) of fish reared in ponds under T_3 were

significantly higher than of those on T_2. Treatment influenced significantly labour and feed costs, fish yield, revenue and gross margin. Fish reared in ponds under T_3 resulted into significantly higher yield (13,065 ± 458 kg/ha/year) and profit (TZS 25,600,813 ± 3,007,007) than those cultured under T_2 and T_1. It is concluded that the combination of weekly fertilization of ponds and concentre feeding at 2.5% of fish biomass promotes higher growth rate and results into higher profit than either weekly fertilization alone or feeding alone at 5% of fish biomass.

INTRODUCTION

In Tanzania aquaculture sector contributes to national food security and economic development, especially of rural poor farmers. Although it accounts only for a small proportion of total fish produced at the national level, aquaculture production provides vital animal protein to the people residing in areas which are located far away from the major fishery resources or where transport of fish is either difficult or too expensive. In some parts of the country where protein intake per capita is low and where protein malnutrition prevails, the socio-economic benefit of aquaculture is high. It provides employment and is a source of income for resource poor farmers. In recent years, there has been a growth in interest on aquaculture, mainly because of decline in capture fisheries. Aquaculture production is practiced by small-scale farmers in the country, mostly in the regions of Arusha, Mbeya, Iringa, Morogoro, Kilimanjaro, Ruvuma, Tanga, Coast, Dar es Salaam, Lindi, and Mtwara. Currently, it is estimated that there are about 21,300 grow-out earthen ponds in operation (MLFD, 2015) and over 95% of the farmers culture Nile tilapia (Oreochromis niloticus) under mixed-sex culture (Kaliba et al., 2006).

The emphasis of the national fisheries policy (URT, 1997) is on a semi-intensive integrated mode of fish culture, focusing on Nile tilapia. (Oreochromis niloticus). Pond culture of Nile tilapia is now viewed as a possible source of livelihoods for farmers residing in proximity to the urban markets of cities and towns. Nile tilapia is given first priority due to its desirable aquaculture characteristics, including 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). The aquaculture sub-sector has a great potential for expansion due to the fact that Tanzania is endowed with ample water resources all over the country. Despite the available potentials for fish production, low production is experienced in many small-scale ponds due to low productivity of the commonly cultured species coupled with poor management. Management practices for pond-cultured tilapia in Tanzania involve only pond fertilization and feeding. Small-scale fish farmers fertilize their ponds using farm yard manure before stocking and apply manure once per month during grow-out (Chenyambuga et al., 2014). Some farmers put manure in cribs in order to fertilize the ponds slowly. Cattle, sheep, goat, and chicken manure are commonly used to fertilize fish ponds. Feeding of pond cultured Nile tilapia depends on natural food and concentrate feeds. Farmers in rural areas provide maize bran, kitchen leftovers, and vegetables/weeds as supplementary feeds to their fish twice per day. The amount provided varies from farmer to farmer. The irregular fertilization and supplementation of low quality feeds results into low production.

Weekly fertilization of fish pond has been shown to increase fish yields by increasing primary productivity through released inorganic nutrients, or by providing organic carbon through heterotrophic pathways (Knud-Hansen et al., 1991). When ponds are fertilized, nutrients stimulate the growth of phytoplankton. Phytoplankton is food for other organisms (zoo-plankton and larger animals) that are eaten by fish. However, pond fertilization with excessive amount of fertilizer can cause severe environmental issues due to high concentration of algae that lead to algal blooms and hypoxic waters. Excess nitrogen input can cause high unionized ammonia concentrations, which may reduce fish growth or cause mortality. Moreover, provision of feeds in excess of what can be taken by the fish leads to wastage of diet and diet waste means deteriorated water quality and economical losses (Ali et al., 2010). This situation often causes mass mortality of tilapia and consequently the farmers abandon fish farming. Sustainable production of tilapia largely depends on quality of water. In the broadest sense, the term water quality includes all physical, chemical, and biological characteristic of water. Pond water characteristics, physical, chemical and biological factors, interact in pond ecosystem as well as the organisms being cultured (Egna and Boyd, 1997). Maintaining proper water quality parameters is very important for survival, growth, and reproduction of aquatic organisms. Water quality in fish ponds is a major factor determining the production of fish (Egna and Boyd, 1997) and needs to be monitored.

Sustainable fish farming depends on the maintenance of healthy pond water environment and the production of sufficient fish food organisms. Among the primary factors limiting the production capacity of a pond is the quantity of available nutrients, which form basic materials for structure and growth of living organisms. Proper pond fertilization and supplementary feeding techniques are used to supply these nutrients in optimal quantities, thereby overcoming natural deficiencies, in order to obtain maximum possible fish yield from a water body. A study done in Bangladesh (Wahab et al., 2014) indicated that proper pond fertilization combined with supplementary feeding at 50% satiation level results in higher fish yield and benefit-cost ratio for polyculture of tilapia and Silver carp than culturing on fertilizers or 100% feeding alone. Another study done in Cambodia (Phanna et al., 2014) showed that pond fertilization plus supplementary feeding (50% satiation) is the most effective feeding for optimization of production, feed conversion ratio, and growth performance of tilapia cultured under semi-intensive system or small-scale aquaculture. In Tanzania information on the combined effects of fertilization and supplementary feeding on water quality parameters in ponds and growth performance of fish is lacking. It is not known which pond management system can be used by small-scale farmers in order to reduce cost and increase production and profit. This study was carried out to compare the effects of pond fertilization alone, concentrate feeding alone at 5% of fish biomass and a combination of fertilization and feeding at 2.5% of fish biomass on water quality parameters and growth performance and yield of Nile tilapia.

METHODOLOGY

Study location and Sampling procedure

A study was conducted at Tindiga Village, Kilosa district, Tanzania. Kilosa district is one of the seven districts of Morogoro region and it is located approximately 300 km inland from Dar es Salaam. The district has an area of 14,245 km^2 and lies between latitude 5°55' S and 7°53' S and longitude 36°30’E and 37°30'E. The district comprises mostly flat lowland and experiences an average of eight months of rainfall (October–May), with the highest levels received between March and April. The rainfall distribution is bimodal, with short rains (November – January), followed by long rains (mid-February –May). Mean annual rainfall ranges between 800 and 1,600 mm. Kilosa district is located at 2200 m above sea level and has mean annual temperature of about 25°C. The district has a total of 164 villages. Tindiga village, which is located 13 km away from Kilosa, town was purposely selected because of existence of project fish farmers. The farmers were trained by the project supported by AquaFish Innovation Lab in 2013 and following the training the farmers constructed fish ponds and started culturing Nile tilapia.

To complement the results obtained from farmer's earthen ponds, another experiment was conducted at Magadu fish farm, Sokoine University of Agriculture (SUA), Morogoro using nine concrete tanks. Sokoine University of Agriculture is located between latitude 6 - 7°S and longitude 37 - 38°E at an altitude of about 500 - 600 m above sea level. The area receives an average annual rainfall of between 600 and 1000 mm. The climate is characterized by bimodal rainfall patterns, with short rains starting in November and ending in December and long rains starting in March and ending in May. The temperature ranges from 25^0 to 30^0C.

Data collection procedure

Two experiments were conducted. The first experiment was conducted using nine earthen ponds at Tindiga Village, Kilosa District, Tanzania. The size of ponds ranged from 160 to 242 m^2 with average pond size of 188.67 m^2. The treatments were three and included weekly fertilization alone with urea and Di-Ammonium Phosphate (DAP) at a rate of 3 g/m^2 and 2 g/m^2, respectively (T_1), concentrate feeding alone at 5% of fish biomass (T_2), and weekly fertilization with urea and DAP plus concentrate feeding at 2.5% of fish biomass (T_3). The treatments were assigned randomly to the fish ponds and each treatment was replicated three times. The concentrate feed comprised of fish meal (25%), cotton seed cake (10%), sunflower seed cake (10%), maize meal (4%), wheat bran (50%) and mineral premix (1%) and had a crude protein content of 30% CP.

Prior to the start of the experiment, all ponds were drained, cleaned to remove decomposed matters, inlets and outlets were rehabilitated, dried for five days and then refilled with water and fertilized with urea and DAP at a rate of 3 g/m^2 and 2 g/m^2, respectively (except those under T_2), and left for 14 days before being stocked with fingerlings. The ponds were stocked on 14th August, 2017 with sex-reversed Nile tilapia with average weight of 1.1 g at a stocking density of 3 fingerlings per m^2. Fingerlings cultured in ponds under T_2 and T_3 were fed 10% and 5% of their body weights, respectively, for the first two months and thereafter the amount of feed was reduced to 5% and 2.5% for T_2 and T_3, respectively. The fish were fed twice per day at 10.00 am and 4.00 pm and the experiment took 166 days.

Fish body weights and lengths were measured at the beginning of the experiment and then after every two weeks. A random sample of 30 fish per pond, 5% of total fish in each pond, was taken for measurement of body weight and length. Weights of individual fish were measured using digital weighing scale, while body length was measured by using a ruler. Pond water pH, dissolved oxygen (DO), conductivity, total dissolved solids, salinity, and temperature were measured in each pond using Multiparameter (HI 98198 PH/EC/DO Multiparameter HANNA instrument). The water samples for measurements for these variables were collected at three depths through the water column (i.e., at the surface (10 cm from the top), middle of the water column, and just off the pond bottom). Dawn measurements were done weekly between 0600 and 0700 hours, while diel measurements were taken three times during the experimental period (i.e., at the beginning, middle, and end of the experiment). Diel measurements were taken nine times in 24 hours at an interval of 3 hours (i.e., at 0600 h, 0900 h, 1200 h, 1500 h, 1800 h, 2100 h, 2400 h, 0300 h and 0600 h). Water samples were collected in 400 ml vials for determination of nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), alkalinity, and total nitrogen. In addition, more water samples were collected in 100 ml vials for determination of chlorophyll a and phosphorus. The water samples were taken weekly from five points in each pond at a depth of 15 cm from the top and mixed together to get composite samples. They were collected between 0900 and 1100 h, put in a cool box and transported to the laboratory at Sokoine University of Agriculture (SUA), where they were stored in the deep freezer until analysis at the end of the experiment. For determination of algae biomass and species composition, two nets, each with a size of 25 cm x 50 cm (1250 cm^2) and mesh size of 20 μm were installed in each pond. Algae samples were collected from each net and put in 400 ml and 5 ml vials. The vials were put in a cool box and transported to the laboratory at SUA, where they were stored in the deep freezer until analysis at the end of the experiment. Water depths were monitored daily by installing in each pond a wooden bar graduated in cm. Water depth was read from the wooden bar daily. Water evaporation rate was measured with an evaporation pan. Water turbidity was determined weekly with a secchi disc with a diameter of 20 cm.

The second experiment was conducted at SUA using nine concrete tanks, each having a surface area of 3.36 m^2. The treatments used in this experiment were the same as those used in the first experiment. The sex-reversed Nile tilapia were stocked at the same stocking rate (3 fingerlings/m^2)

and fed the same feed at the same feeding levels and frequency used in the first experiment. All fish in each tank were individually measured for body weight and length before the start of the experiment and then every two weeks thereafter. Measurement of water quality parameters followed the same protocol as that used in the first experiment.

At the end of each experiment, the following parameters were computed as shown below:
(i) Growth rate (GR)

$$GR = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Time in days}} \quad (1)$$

(ii) Specific growth rate (SGR)

$$SGR = \frac{[\text{In(Final weight (g)} - \text{In(initial weight (g)}]}{\text{Time in days}} \text{ X } 100 \quad (2)$$

(iii) Feed convertion ratio (FCR)

$$FCR = \frac{\text{Total weight of food consumed(g)}}{\text{Total weight gain by fish(g)}} \quad (3)$$

(iv) Survival rate (SR)

$$SR = \frac{\text{Total number stocked-total number died}}{\text{Total number stocked}} \text{ x } 100 \quad (4)$$

(v) Annual Yield (AY)

$$AY = \frac{\text{Total weight of fish harvested x 365}}{\text{Experimental period in days}} \quad (5)$$

(vi) Gross margin
Gross margin (GM) analysis was used to determine profitability of fish culture in each treatment and was calculated as

$$GM = \text{Total revenue from fish sold fish} - \text{Total variable costs} \quad (6)$$

Statistical analysis
Data generated on growth performance parameters (GR, SGR, FCR and yield) and water quality parameters (pH, Temperature, DO, Total Dissolved Solids (TDS), Salinity, turbidity) were analyzed using R Studio software version 3.4.4. Analysis of variance was done in a completely randomized design and the effect of treatment was tested using the F test. Tukey's test was used to determine the significance of the differences between a pair of treatment means.

RESULTS

Water quality parameters in earthen ponds and concrete tanks subject to three treatments
Table 1 shows mean ± se of water quality parameters (pH, DO, temperature, conductivity, TDS, salinity and turbidity (secchi disk reading)) in earthen ponds while Table 2 shows the mean ± se for the same water parameters in concrete tanks used in the experiment. In both culture system (earthen ponds and concrete tanks) treatment had significant effect on water pH, DO, conductivity, TDS and salinity but not on temperature and turbidity. Ponds under fertilization alone (T_1) and fertilization plus feeding (T_3) had higher ($p \leq 0.001$) water pH than those under the treatment of concentrate feeding alone (T_2). Ponds under T_1 had higher ($p \leq 0.001$) DO values than those under T_2 and T_3, but there was no significant difference between the DO values in ponds under T_2 and T_3. On the other hand ponds subjected T_2 had higher ($p \leq 0.001$) conductivity, TDS and salinity than those under T_1 and T_2. For concrete tank system, tanks under T_1 had significantly higher pH and DO than those under T_2 and T_3. Moreover, tanks under T_3 had significantly higher pH and DO compared to those on T_2. For

conductivity and TDS, the values obtained in tanks under T_1 and T_2 did not differ significantly but were significantly different from the values obtained in tanks under T_3. Salinity and temperature values did not differ significantly among the treatments.

Table 3 shows mean (± se) of diel water quality parameters in pond water under the three treatments. The results show that treatment had significant effects on water pH, DO and conductivity. Water pH in ponds under fertilization alone (T_1) differed significantly from that obtained in ponds under feeding alone (T_2), but was not significantly different from that obtained in ponds under a combination of fertilization and feeding (T_3). Similarly, the pH values in ponds under T_2 were not significantly different from that in ponds under T_3. The DO values differed significantly among the treatments. Ponds under T_1 showed the highest while those on T_2 had the lowest DO values. Water in ponds under T_2 showed higher conductivity values than that in ponds under T_3, which, in turn, had higher conductivity values than that in ponds under T_1. The mean values for water salinity, TDS and temperature did not differ among the treatments.

The diel measurements for water quality parameters in concrete tanks are shown in Table 4. The results show that treatment significantly affected water pH, DO, conductivity and TDS but not salinity, and temperature. Water in tanks under T_1 had higher pH values than that in tanks under T_2 and T_3, but water pH values in T_2 and T_3 were not significantly different. The water DO value in tanks under T_1 was significantly higher than that in tanks under T_3, which in turn was higher than that in tanks under T_2.

Figure 1 and Figure 2 show the mean DO values for a period of 24 hours in pond water and concrete tank water, respectively. In both ponds and concrete tanks, the highest DO values were observed under T_1 while the lowest values were found under T_2. Also in both systems, the highest DO values were obtained at 1500 hours while the lowest values were observed between midnight and 0600 hours. With regard to temperature, the highest values were obtained at 1500 hours while the lowest values were observed from 0300 hours to 0600 hours (Figure 3 and Figure 4). Figure 5 and Figure 6 show the mean pH values for a period of 24 hours in both earthen ponds and concrete tanks. In both systems, like for ponds, the highest DO values were observed under T_1 while the lowest values were found under T_2. Similarly, peak pH values were observed at 1500 hours while the lowest values were observed from 0300 to 0600 hours. It seems that DO, pH and temperature values followed the same pattern, i.e. they were lowest at 0600 hours, start to increase at 0900 hours and reached the peak at 1500 hours and then started to decrease and reached the lowest values at 0300 hours.

Table 1: Comparison of water quality parameters in pond under three different treatments

	Treatment			
Water variable	**Fertilization alone**	**Feeding alone**	**Fertilization and feeding**	***p*-Value**
pH	$8.23{\pm}0.02^{a}$	$8.08{\pm}0.02^{b}$	$8.20{\pm}0.02^{a}$	4.909e-07
DO(mg/L)	$4.43{\pm}0.11^{a}$	$3.88{\pm}0.09^{b}$	$4.03{\pm}0.11d^{b}$	0.0007
Conductivity(µS/cm)	$1324{\pm}9^{b}$	$1363{\pm}8^{a}$	$1343{\pm}9^{ab}$	0.0051
TDS	$662{\pm}4^{b}$	$681{\pm}4^{a}$	$672{\pm}4^{ab}$	0.0052
Salinity(PSU)	$0.66{\pm}0.0^{c}$	$0.68{\pm}0.0^{a}$	$0.67{\pm}0.0^{b}$	0.0072
Temp (^{O}C)	$25.20{\pm}0.09^{a}$	$25.24{\pm}0.09^{a}$	$25.30{\pm}0.08^{a}$	0.6918
Water depth(cm)	$31.5{\pm}0.2^{c}$	$34.8{\pm}0.3^{a}$	$33.5{\pm}0.2^{b}$	2.2e-16
Secchi disk (cm)	$25.7{\pm}0.7^{a}$	$25.7{\pm}0.7^{a}$	$24.3{\pm}0.7^{a}$	0.2848

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.001$.

Table 2: Comparison of water quality parameters in concrete tanks subjected to three different treatments

Water variable	Treatment T1	T2	T3	p-Value
pH	8.35±0.05[a]	7.54±0.06[c]	7.76±0.05[b]	2.2e-16
DO(mg/L)	9.97±0.21[a]	6.51±0.20[c]	7.84±0.20[b]	2.2e-16
Conductivity(µS/cm)	81±1[a]	79±1[a]	76±1[b]	0.0001
TDS	41±0[a]	40±1[a]	38±1[b]	1.949e-05
Salinity(PSU)	0.04±0.0[a]	0.04±0.0[a]	0.03±0.0[b]	0.0004
Temp (^{O}C)	27.53±0.11[b]	27.61±0.10[ab]	27.82±1.10[a]	0.1037

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.001$.

Table 3: Diel water quality parameters of pond water under three different treatments

Water variable	Treatment T1	T2	T3	*p*-Value
pH	8.82±0.08[a]	8.54±0.04[b]	8.68±0.06[ab]	0.0075
DO(mg/L)	6.77±0.22[a]	5.14±0.18[c]	6.05±0.23[b]	6.785e-07
Conductivity(µS/cm)	1323±7[c]	1409±4[a]	1350±7[b]	2.2e-16
TDS	662±4[a]	704±2[a]	713±36[a]	0.1867
Salinity (PSU)	0.65±0.0[a]	0.70±0.0[a]	0.71±0.0.04[a]	0.2376
Temp(^{O}C)	30.17±0.31[a]	30.22±0.27[a]	29.99±0.25[a]	0.8191

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.001$.

Table 4: Diel water quality parameters in concrete tanks under three different treatments

Water variable	Treatment T1	T2	T3	p-Value
pH	9.61±0.06[a]	8.30±0.05[b]	8.38±.06[b]	2.2e-16
DO(mg/L)	8.37±0.12[a]	4.87±0.15[c]	5.39±0.18[b]	2.2e-16
Conductivity(µS/cm)	53±1[c]	70±1[a]	64±1[b]	2.2e-16
TDS	26±0[c]	35±1[a]	33±1[b]	2.2e-16
Salinity (PSU)	0.02±0.0[b]	0.03±0.0[a]	0.03±0.0[a]	5.736e-06
Temp(^{O}C)	26.72±0.11[b]	27.06±0.11[a]	27.08±0.11[a]	0.0263

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.001$.

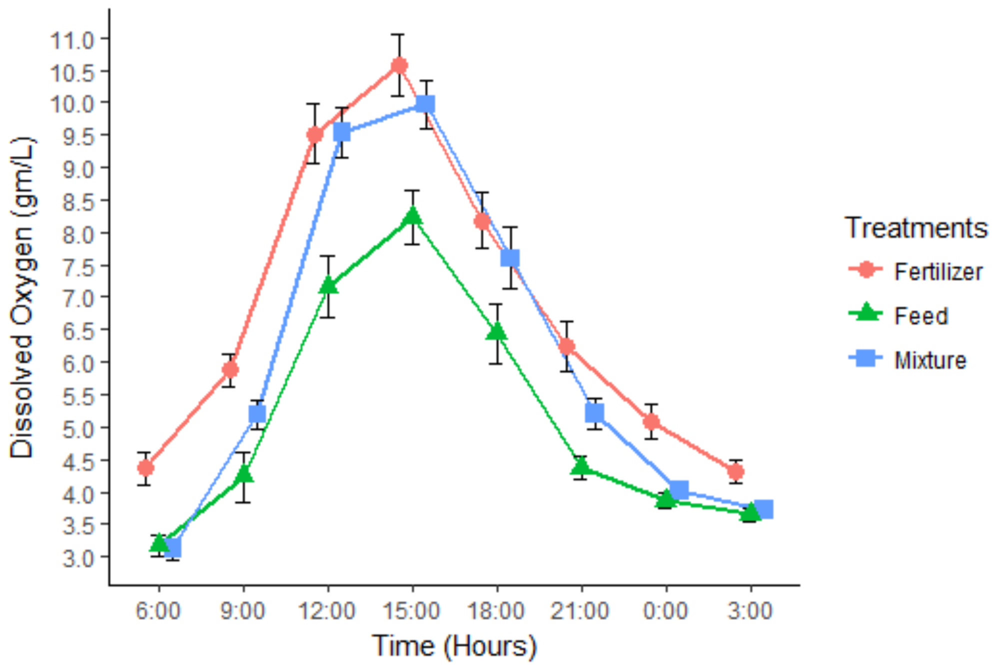

Figure 1: Diel mean DO values in pond water under the three different treatments

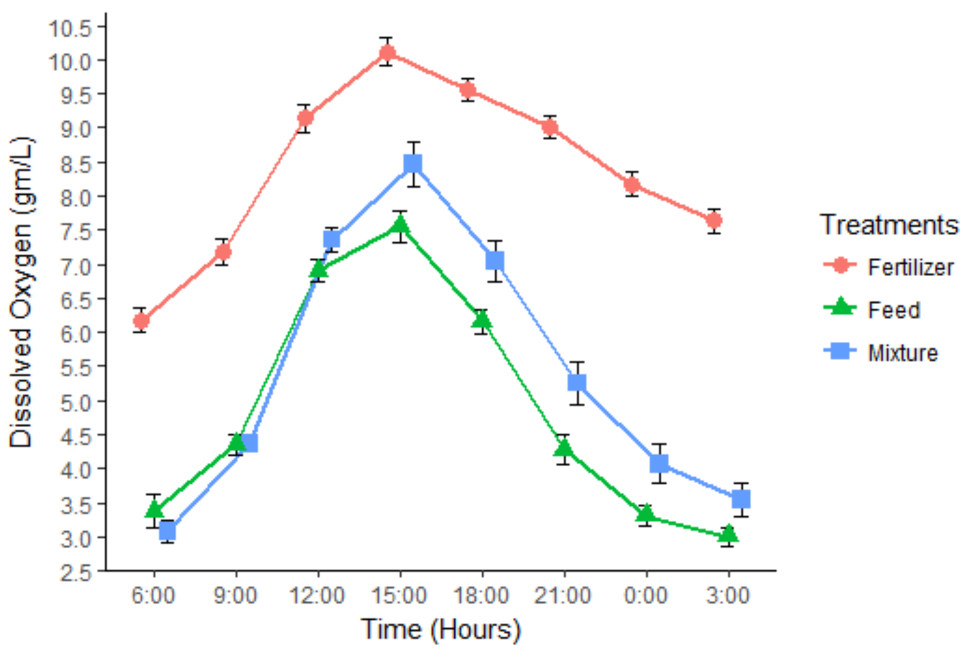

Figure 2: Diel mean DO values in concrete tanks under the three different treatments

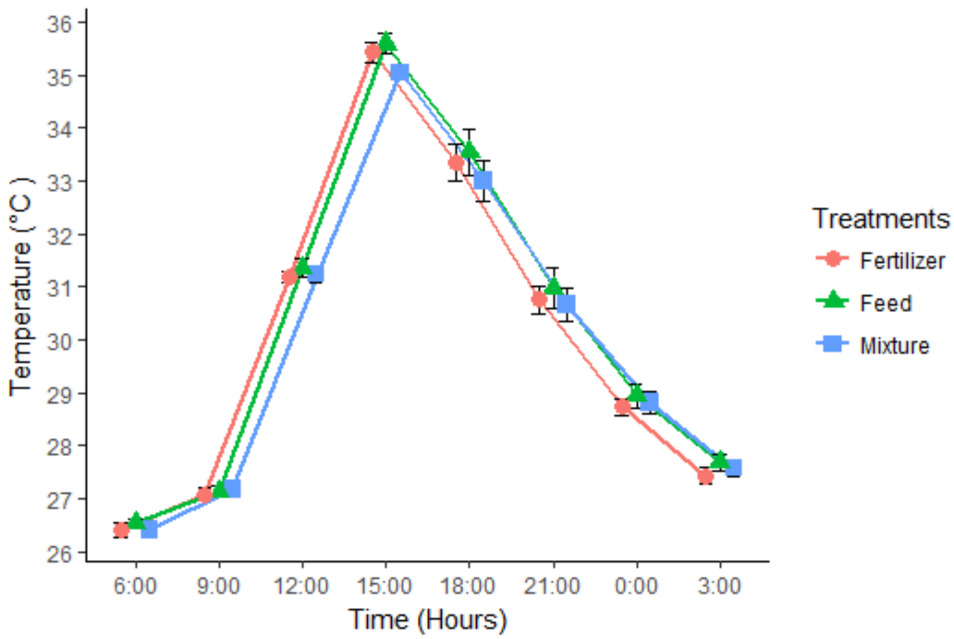

Figure 3: Diel mean water temperature in ponds under three different treatments

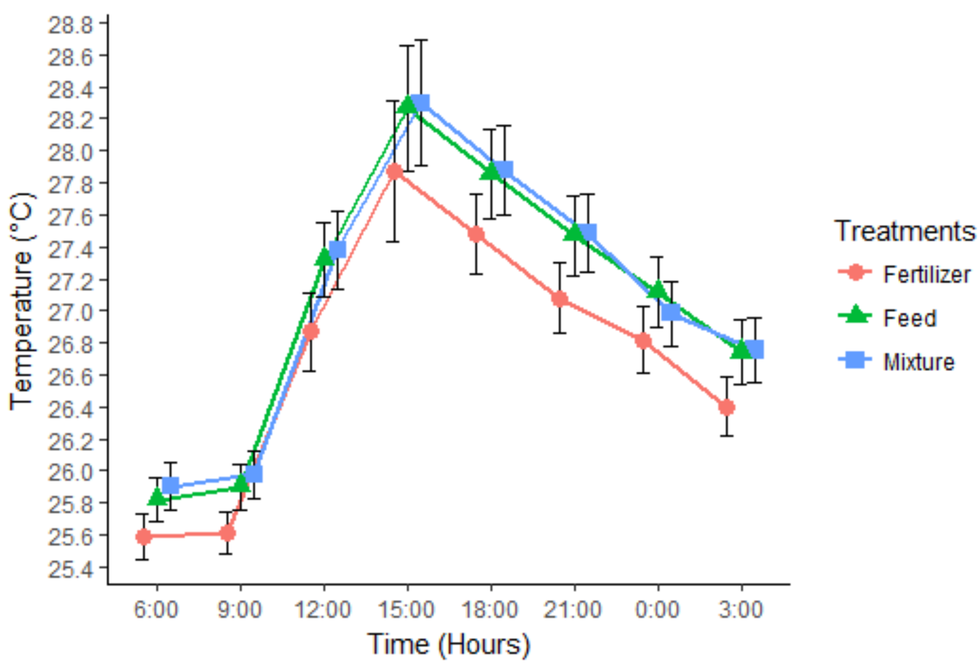

Figure 4: Diel mean water temperature in concrete tanks under three different treatments

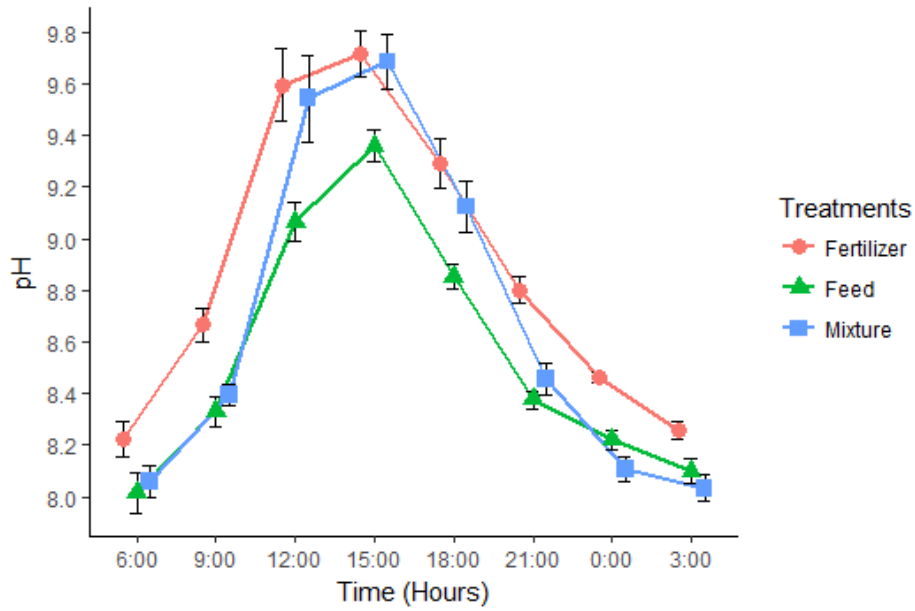

Figure 5: Diel mean water pH in ponds under three different treatments

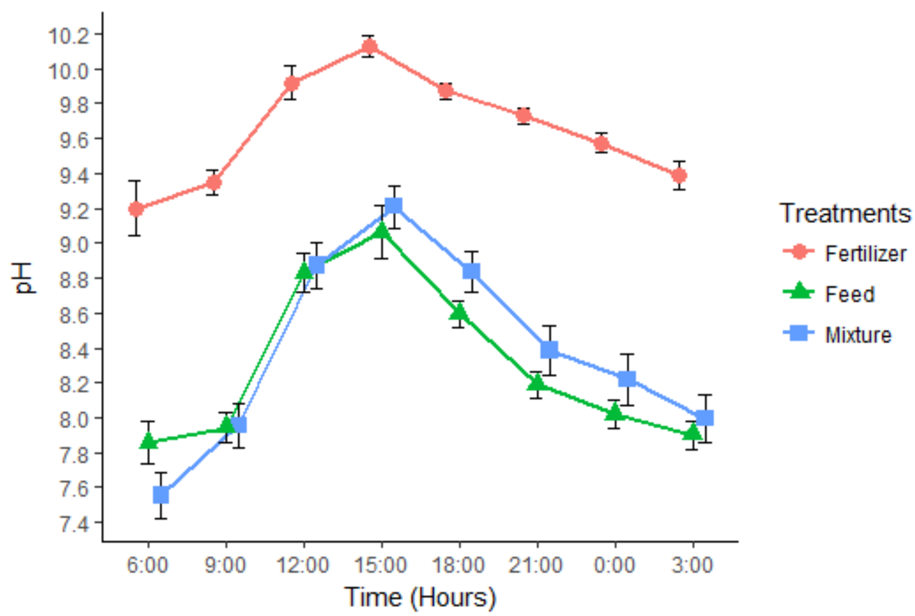

Figure 6: Diel mean water pH in concrete tanks under three different treatments

Growth performances of Nile tilapia (O. niloticus) cultured in earthen ponds and concrete tanks under three treatments

Table 5 and Table 6 show the growth performance, feed conversion ratio and survival of Nile tilapia (*O. niloticus*) culture in ponds and concrete tanks, respectively, under three treatments (fertilization alone (T_1), feeding alone (T_2) and a combination of fertilization and feeding (T_3)). In both earthen ponds and concrete tanks, treatment had significant effects on final body weight, weight gain, growth rate, specific growth rate and feed conversion ratio (FCR), but did not significantly affect survival rate. The results shown in Table 5 indicate fish culture in earthen ponds under T_1 had significantly lower final body weight, weight gain, growth rate and specific growth rate than those cultured in ponds under T_2 and T_3. Fish culture in ponds under T_2 on average gained 95.4 g while those on T_3

gained 128.4 g more weight than those on T_1. On the other hand the mean final body weight, weight gain, growth rate and specific growth rate of fish on T_3 were significantly higher than of those on T_2. The body weight gain and growth rate of fish reared in ponds under T_3 exceeded those of fish reared in ponds under T_2 by 33 g and 0.2 g/day, respectively. Moreover, the fish under T_3 had significantly lower FCR than those on T_2, mainly because they were given half the amount of feed provided to the fish under T_2 but they grew faster compared to those under T_2. Survival rate of fish did not differ ($p > 0.05$) among the treatments and ranged from 89.6 to 90.0% in ponds under T_2 and T_1, respectively. Like under pond system, fish cultured in concrete tanks under T_1 had significantly lower final body weight gain, weight gain, growth rate and specific growth rate than those reared in concrete tanks under T_2 and T_3. Fish reared in concrete tanks under T_3 had higher final body weight gain, weight gain, growth rate and specific growth rate and lower feed conversion ratio than those under T_2. The growth performance of fish for the whole experimental period are shown in Figure 7 for earthen ponds and Figure 8 for concrete tanks. In both systems, fish culture under T_3 showed the highest performance, followed by those under T_2. The fish reared under T_1 had the lowest growth performance. When comparison is made between earthen ponds and concrete tanks, it can be seen that fish under T_1 gained more weight in ponds than in concrete tanks while those reared under T_2 and T_3 gained more weight in concrete tanks than in earthen ponds.

The results for economic analysis are shown in Table 7 for fish reared in earthen ponds under the three treatments. Among the variable costs, fingerling costs did not differ ($p > 0.05$) among the treatments while labour and feed costs differed significantly among the treatments. Ponds under T_3 had the highest labour costs, while those under T_1 had the lowest. The highest feed cost was observed under T_2. Feed cost accounted for 60.03% and 40.85% of total variable costs in treatment T_2 and T_3, respectively. Fertilizer cost did not differ ($p > 0.05$) between the ponds under T_1 and T_3, mainly because the same amount of fertilizer was used in the two treatments. Treatment influenced significantly the yield, revenue and gross margin. Fish reared in ponds under T_3 resulted into significantly higher yield, revenue, and profit than those cultured under T_2 and T_1. Fish reared under T_2 had higher yield and revenue than those under T_1. However, fish reared under T_1 resulted into profit while those under T_2 resulted into a loss mainly because of the higher feed cost.

Table 5: Comparison of growth performance of Nile tilapia (*O. niloticus*) cultured in ponds under three different treatments

Growth variable	Treatment			*p*- Value
	T1	**T2**	**T3**	
Initial body weight (g)	1.3 ± 0.1^{a}	1.0 ± 0.1^{b}	0.9 ± 0.0^{b}	0.003
Final body weight (g)	66.7 ± 1.1^{c}	161.8 ± 3.6^{b}	194.8 ± 4.5^{a}	0.000
Weight gain (g)	65.4 ± 1.1^{e}	160.8 ± 3.6^{b}	193.8 ± 4.5^{a}	0.000
Growth rate (g/day)	0.4 ± 0.0^{e}	1.0 ± 0.0^{b}	1.2 ± 0.0^{a}	0.000
Specific growth rate (%)	2.5 ± 0.0^{c}	3.1 ± 0.0^{b}	3.3 ± 0.0^{a}	0.000
Survival (%)	90.0 ± 0.0^{a}	89.6 ± 0.2^{a}	89.7 ± 0.4^{a}	0.4672
FCR	-	4.1 ± 0.3^{a}	2.0 ± 0.1^{b}	0.0085

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.001$.

Table 6: Comparison of growth performance of Nile tilapia (*O. niloticus*) cultured in concrete tanks under three different treatments

Growth variable	Treatment			*p*- Value
	T1	**T2**	**T3**	
Initial body weight (g)	1.5 ± 0.1^{a}	1.0 ± 0.1^{b}	1.4 ± 0.1^{a}	0.0011
Final body weight (g)	42.3 ± 2.8^{c}	230.0 ± 7.5^{b}	257.6 ± 4.5^{a}	0.000
Weight gain (g)	40.9 ± 2.8^{c}	229.0 ± 7.5^{b}	256.2 ± 4.5^{a}	0.000
Growth rate (g/day)	0.2 ± 0.0^{c}	1.4 ± 0.0^{b}	1.6 ± 0.0^{a}	0.000
Specific growth rate (%)	2.0 ± 0.1^{c}	3.3 ± 0.1^{a}	3.2 ± 0.1^{b}	0.000
Survival (%)	90 ± 0.0^{a}	87.7 ± 2.3^{a}	90 ± 0.0^{a}	0.4219
FCR		3.3 ± 0.2^{a}	1.5 ± 0.0^{b}	0.0016

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.001$.

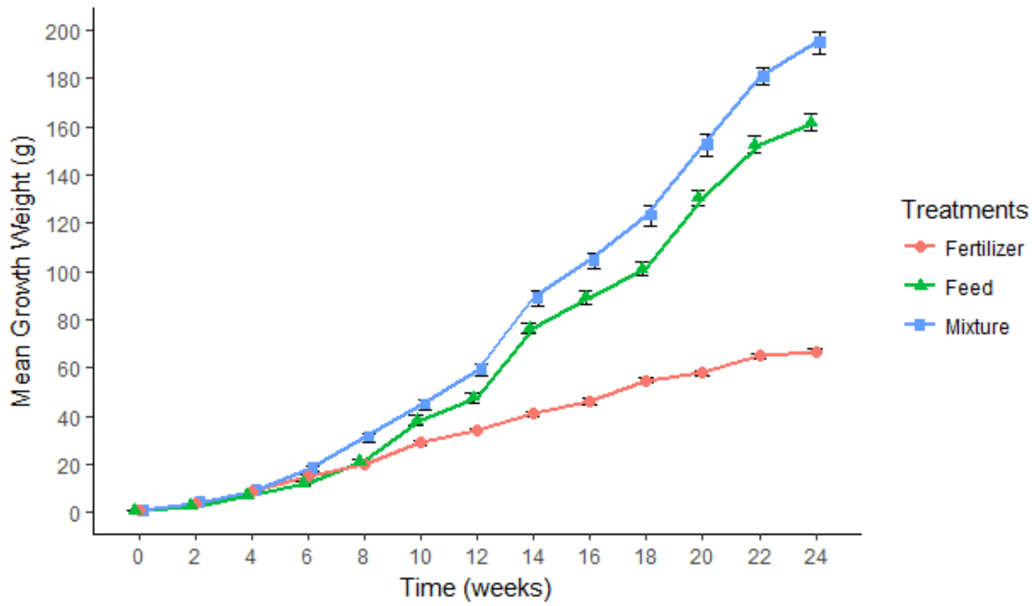

Figure 7: Comparison of growth performance of *O. niloticus* cultured in earthen ponds under three different treatments

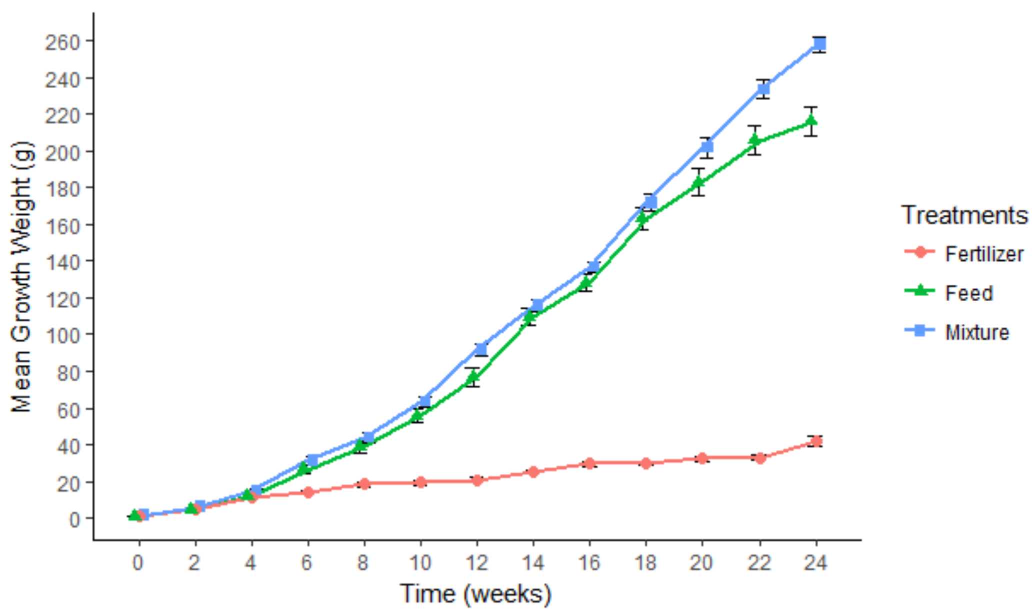

Figure 8: Comparison of growth performance of *O. niloticus* cultured in concrete tanks under three different treatments

Table 7: Comparison of variable costs, revenue and profit obtained from *O.niloticus* cultured in earthen ponds under three different treatments.

VARIABLE	Treatment			*p*-Value
Variable costs (TZS /ha/Year)	**T1**	**T2**	**T3**	
Fingerling Cost	22,958,144±601,234[a]	22,198,993±1,009,160[a]	22,484,390±1,364,821[a]	0.8383
Labour cost	1,654,749±48,483[b]	14,279,749±6,009,183[ab]	23,992,930±143,720[a]	0.0337
Fertilizer cost	208,187±4,096[a]	-	202,498±4,554[a]	0.4319
Feed cost	-	54,796,586±5,683,682[a]	32,233,886±572,883[b]	0.0446
Total cost	24,821,080±560,314[b]	91,275,328±12,580,773[a]	78,913,704±652,772[b]	0.0046
Algae Dry matter(g)	34.6±0.8[a]	36.0±1.0[a]	36.0±1.1[a]	0.5028
Productivity				
Yield (kg/ha/year)	4,602±376[b]	10,720±962[a]	13,065±458[a]	0.0010
Total revenue(TZS/ha/year)	36,820,392±3,008,558[b]	85,763,684±7,696,247[a]	104,514,517±3,659,779[a]	0.0010
Gross margin	11,999,312±2,588,720[ab]	(5,511,645)±8,013,280[b]	25,600,813±3,007,007[a]	0.0373

Note: Means with the same superscript letter in the same row do not differ while those with different superscript differ significantly at $P \leq 0.05$.

CONCLUSION

This study has shown that a combination of weekly fertilization and supplementary feeding is better than feeding alone or fertilization alone. From the results of this study the following conclusions can be made.

i. Combination of weekly fertilization of ponds and concentre feeding at 2.5% of fish biomass promotes higher growth rate and results into higher profit than either weekly fertilization alone or feeding alone at 5% of fish biomass.
ii. Combination of weekly fertilization of ponds and concentre feeding at 2.5% of fish biomass does not affect water quality parameters beyond the range recommend for tilapia growth.
iii. Water quality parameters (pH, DO, salinity and temperature) in ponds are lowest at 0300 - 0600 hours and attain peak value at 1500 hours.

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Optimizing the Use of Commercial Feeds in Semi-Intensive Pond Production of Tilapia in Ghana; From Nursery to Grow-Out

Production System Design and Best Management Alternatives/Experiment/16BMA02PU

Emmanuel A. Frimpong[1], Regina Edziyie[2], Stephen Amisah[2], Kwasi A. Obirikorang[2], and Kwamena Quagrainie[3]

[1]*Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University, 310 West Campus Dr, Blacksburg, Virginia 24061, USA*
[2]*Department of Fisheries and Watershed Management, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[3]*Purdue University, Indiana, USA*

ABSTRACT

Pond-based tilapia production in Ghana still suffers from the absence of a viable nursery sector and high cost of production due to high feed cost and suboptimal use of fertilization and feeding strategies. This investigation used a series of experiments and economic analyses to determine areas where both nursery and grow-out feeds and feeding can be optimized as well as make the feed ingredients local, sustainable, and low-cost. Experiments were carried out at the Kwame Nkrumah University of Science and Technology fish farm, the Pilot Aquaculture Center of the Fisheries Commission, and a private fish farm, all in Kumasi, Ghana. In the first experiment we showed that a grow-out feed with 25% crude protein (CP) results in the same growth in fertilized ponds as the standard 23% CP feed on the market while costing approximately 15% less. Therefore, reduced protein feed would result in substantial profitability of tilapia grow-out operations. In the second experiment, we showed that feeding tilapia on alternate days or daily at half ration both result in substantial reduced cost of production not offset by slightly reduced growth, compared with feeding daily at full ration. The two treatments resulted in approximately triple and double, respectively, of the return on investment when compared with the standard practice. The remaining studies considered nursery production systems and comprised three successive experiments to determine the optimum stocking density and growth performance of Nile tilapia fingerlings using both commercial and experimental feeds. Additionally, zooplankton consumption was studied and represented an important part of the fish's diet. In the first of the nursery experiments commercial feed (48% CP) was used to raise 2-g tilapia in 1m^3 hapas in ponds to >20g. Four stocking densities were used; 28/m^3, 55/m^3, 83/m^3 and 110/m^3 and growth observations were made10, 20, 30 and 40 days after the experiment. In the second nursery experiment, experimental feeds (48% CP) in three iso-nitrogenous and iso-caloric diets were formulated and compared with the commercial diet used in earlier. A digestibility experiment was carried out in 120L indoor tanks and ammonia excretion rates were determined. For the third experiment, the best two diets from the second experiment were field tested along with the commercial diet. Stocking density was 100/1m^3 in 1m^3 hapas in a pond and the experiment lasted for 40 days with sampling done every 10 days. In all cases, survival rates, feed conversion ratio (FCR), growth performance and water quality variables were monitored. For the last experiment, gut content analysis was also conducted. For the first nursery experiment, survival rates ranged from 77-85% and decreased slightly but significantly with stocking density. FCR was below 1 for all treatments and averaged 0.67 ± 0.02 standard error. Mean final weight did not differ among the treatment at the end of the 40 days. For the digestibility experiment, fecal matter output was highest for diets 2 and 3 (relatively higher fiber content) and differed from the control (commercial feed). Protein and dry matter digestibility were similar for all diets. Diet 3 however, had a lower lipid digestibility compared with the others. The FCR was similar among the diets with a low of 1.61 ±0.17 (sd) in the Diet 2 and a high of 1.79 ± 0.12 (sd) in diet 3 with a survival rate ranging between 83-97%. For the last nursery

experiment (field testing), survival rates were 75-92%. There were no significant differences between the mean weights of the fingerlings harvested among the different diets. FCR ranged from 0.55 in the Control to 0.65 in Diet 3. Fish fed Diet 2 consumed more zooplankton than the control, and diet 3. The most common zooplankton in the diets for all treatments were the rotifers, especially *Brachionus spp*. This investigation shows that numerous opportunities exist from nursery through grow-out in the practice of tilapia farming in Ghana, to reduce feed and feeding cost, increase profitability, and sustainably increase tilapia production in the country.

INTRODUCTION

The small-scale pond sector of the aquaculture industry in Ghana is still struggling to effectively integrate routine and profitable use of commercial floating feeds into farming operations, despite a growing availability of high-quality commercial feeds in the country. Recent AquaFish Innovation Lab studies of growth performance of Nile tilapia (*Oreochromis niloticus*) during the grow-out phase in Ghana showed the superiority of commercial floating feeds compared to farm-made sinking feeds (Ansah and Frimpong 2015). Most farmers already acknowledge that commercial feeds would increase profitability during grow-out, hence a growing number of small-scale tilapia farms have tried commercial floating feeds or use them routinely in their production. For pond-based semi-intensive tilapia farms in Ghana using commercial feeds, feed constitute approximately 60% of the cost of production (Frimpong and Anane-Taabeah 2017). Due to importation of feeds or feed ingredients, this component of cost increases as the value of the local currency depreciates continually against major world currencies.

A combination of high feed cost and lack of technical know-how in the efficient use of feeds and optimal fertilization has been identified as an obstacle to increased yield and profitability of small-scale fish farming in Ghana. Secondly, in spite of modest progress made in the development of an improved strain of Nile tilapia in Ghana, pond farmers scattered through the regions still face a lack of access to all-male fingerlings of the appropriate size for expanded production. Since demand for fingerlings exceeds its supply and the fingerling size and sex reversal needs for cage and pond farms differ, most pond farms can only access fingerlings from the wild or are forced to purchase fingerlings that are too small (fry) and not properly sex-reversed, leading to high mortality, high in-pond breeding, and ultimately, low productivity. Overall throughout the country, not only are hatchery capacities limited but also no true nurseries exist. Therefore, high demand for fingerlings have driven down the size of fingerlings that hatcheries are able to produce and buyers are willing to take, leading to the sale of increasingly smaller sizes of fingerlings that are more susceptible to mortality when handled.

A practical solution for pond farmers is to buy fry at 1.5 - 2g and transport long distances. Others acquire broodstock and produce their fry on-farm. Techniques are needed for farmers to raise fry on quality feed to rapidly reach the sizes at which the tilapia sexes can be identified visually (15-30g) and separated before sexual maturity. For small-scale, pond-based farming, manual separation of the tilapia sexes for stocking continue to be the most viable approach for monosex grow-out since hormonal sex-reversal continue to deliver unacceptably low success rates (70% - 90% male) in numerous trials. Profitable on-farm nursery of tilapia will require optimization of growth rate, survival rate, and feed conversion, whether undertaken with commercial or farm-made feeds.

Due to high protein content, nursery feeds available in Ghana (~48% CP) cost twice as much as grow-out feeds (~30% CP). For both nursery and grow-out feeds, there is a need for sustainable and cost-saving technologies; including reductions in protein content and replacement of fishmeal with alternate, locally available protein sources (Hasan 2017; El-Sayed 2018). Feed development therefore remains critical in the successful establishment of commercial nurseries and pond-based grow-out. Agbo et al. (2011) and Obirikorang et al. (2015) have shown that various agro-by products in Ghana can be a good supplementary protein source in formulating tilapia diets. In this proposed study, a series of experiments and economic analyses were performed to develop a locally verified base of knowledge on cost-saving feeds and feeding strategies for pond-based tilapia producers in Ghana.

OBJECTIVES

1. To evaluate the survival, feed conversion, and growth of Nile tilapia raised in ponds on high-protein (48%) commercial feeds from fry to fingerling sizes.
2. To compare the growth and yield of Nile tilapia in grow-out ponds fed full ration on 30%, 28%, and 25% protein on commercial feeds in combination with fertilization.
3. To compare growth and yield of Nile tilapia in grow-out ponds with 30% protein commercial feeds at full ration, half ration, and alternate-day full ration, in combination with fertilization.
4. To evaluate the palatability/acceptability, digestibility of experimental diets made from locally available ingredients fed to Nile tilapia from fry to fingerling sizes.
5. To evaluate the survival, feed conversion, and growth performance of Nile tilapia raised in ponds on experimental diets from fry to fingerling sizes.
6. To determine the zooplankton composition and importance in fingerling diet.
7. To assess the profitability of the different feeds and feeding strategies for the nursery and grow-out scenarios.

MATERIALS AND METHODS

Study Area

Nursery experiments were carried out in aquarium tanks and hapas mounted in ponds at the Fish Farm at Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Grow-out experiments were conducted in ponds at KNUST farm, Pilot Aquaculture Center of the Fisheries Commission (PAC), and a private farm in Kumasi.

Source of Fish and Water

Well water was used for all studies. Fish for the study was sourced from PAC and a private hatchery in Akosombo, Eastern Region of Ghana. Fish estimated for stocking hapas or ponds were packaged separately in well well-oxygenated plastic bags to prevent over-handling. The fish were acclimatized to the study conditions before each experiment was conducted. For grow-out experiments, fry (about 2g) were first raised in fertilized ponds in hapas on high protein commercial feeds to a target size of 30-40g. Females were then visually identified and removed before stocking males in open ponds at desired densities of approximately $2/m^2$.

Pond Preparation

Experimental ponds ranged in size from $150m^2$ to $630m^2$. Ponds were drained, dried and limed at a rate of 1kg agriculture lime per $10m^2$ to improve alkalinity and ensure that fish eggs from nearby rivers were eliminated. Ponds were then filled and fertilized. Fertilization was achieved with mono ammonium phosphate (MAP) at 2g per m^2 and urea at 3g per m^2; strictly according to the phytoplankton abundance, using Secchi-disk depth as a proxy for abundance. The right proportions of MAP and urea for all the ponds were measured into a container, dissolved with approximately 15 liters of pond water and fertilizer solutions broadcast over the surface of pond.

Experimental Design

Objectives 1-6 are respectively matched with experiments E1, E2, E3, E4, E5, and E6.

Experiment E1 & E5

Experiment E1 & E5 followed a completely randomized design with two factors: 1) Density at 28, 55, 83, and $110/m^3$, and 2) Time (i.e. number of days till fish are observed) at 10, 20, 30, and 40 d (Figure 1). Each treatment was replicated 3 X for a total of 48 experimental units. Each experimental unit is a hapa of size 1m^3 in a pond of >1 m water depth. All fish were stocked at the same size of approximately 2g and fed twice daily, targeting a feeding rate of 10% average body weight (ABW) through 30d and switching to 8% ABW through 40d. Experiment E1 fish were fed on a 48% CP commercial feed, whereas E5 was repeated for each of the best two performing 48% CP experimental feeds (described later) with the commercial feed as a control. For E5, fish were conditioned for one week and the initial weight of the fish used became 3.4g. Only the stocking density of 100/m^3 was used for E5 and the feeding rate was maintained at 8% throughout the experiment. Fish sampling was done every ten days till the end of the experiment after which the quantities of feed fed were adjusted. Physicochemical water quality variables; temperature, DO, pH, and Secchi depth were monitored throughout the experiment.

Density	**28 fry/m^3**				**55 fry/m^3**				**83 fry/m^3**				**110 fry/m^3**			
Days till Observed	10d	20d	30d	40d	10d	20d	30d	40d	10d	20d	30d	40d	10d	20d	30d	40d
	10d	20d	30d	40d	10d	20d	30d	40d	10d	20d	30d	40d	10d	20d	30d	40d
	10d	20d	30d	40d	10d	20d	30d	40d	10d	20d	30d	40d	10d	20d	30d	40d

Figure 1.- Experimental design for experiment E1.

Experiment E2

The first round of Experiment E2 was carried out in six 150-200m^2 experimental ponds at KNUST farm and nine 450-630m^2 at a private farm in Kumasi. One of the larger ponds was lost as an experimental unit due to a flood that allowed an unknown number of adult female tilapia to enter the pond after stocking. The second round was run in nine 250-450m^2 ponds at PAC and six 450-600m^2 ponds at a private farm in Kumasi. This was a one-factor experiment at 3 levels of crude protein (30%, 28%, and 25% protein) following a completely randomized design. The 30% and 28% protein feeds were available on the market in Ghana from a locally-based feed producer. The 25% feed was produced under a special arrangement with the producer and primarily involved replacement of protein with starch. Fish for this experiment was first be stocked as 2g fry in a hapa within each pond and fed on high-protein feeds until they attained approximately 30-40g before being sorted by size and sex for stocking all males at approximately 2/m^2 in experimental ponds. Thereafter, feeding was done two times a day (approximately 10:00am and 4:00pm) starting at 3% ABW/day and reducing through the growth period to 2% ABW/day at experiment termination. For each trial fish growth was monitored for at least 12 weeks to allow growth differences to be clear among treatments.

Experiment E3

Experiment E3 was carried out in nine 350m^2 ponds at PAC. This was a one-factor experiment at 3 levels of feeding (full ration & 30% protein (control); half ration & 30%; alternate day & 30%) following a completely randomized design with 3 replicates per treatment. Stocking, feeding and monitoring followed protocols described for experiment E2.

Experiment E4

This experiment was carried out in a modified Guelph system, with 12 cylindro-conical tanks of volume 150L each and a reservoir tank. Water temperature was maintained at ~25ºC at a water exchange rate of 100Lh^{-1} and tanks were well aerated (≥70% dissolved oxygen saturation) and a light regime of 12 hour light: 12 hour dark throughout the experiment. Each tank was fitted with a sedimentation column at the bottom which allowed for ease of feces collection. Four treatments; one a commercial diet (same as in experiment 1) and three experimental diets were tested. All diets were iso-nitrogenous (crude protein – 48%) and iso-caloric diets (gross energy – 17kJg^{-1}). The treatments were randomly assigned to the experimental units in triplicates and the variables monitored were feed acceptability (using feed intake as a proxy), fecal output, and nutrient digestibility.

Experimental Feeds

The three feeds were formulated using Winfeed Ver 2.8 (Table A1[1]). The protein sources used were agro by-products (soybean meal, and fermented copra meal) and fishmeal. The copra meal was fermented with oyster mushroom *(Pleurotus ostreatus*) following methods described by Agbo and Prah (2014) to help reduce the fiber and lipid contents while improving the overall protein content. The carbohydrate and lipid sources in the diet were wheat bran and palm oil respectively. A vitamin/mineral premix was also added to each diet with tapioca added on as a binder. All ingredients used in diet preparation were finely ground, sieved, weighed at pre-determined quantities and thoroughly mixed with hot water and other ingredients and pelletized using a Bosch meat grinder model MFW67440 with 2mm die plate. The diets were then oven dried for 48 hours at a temperature of 40ºC, cooled and pellets separated by hand.

Fish Stocking and Feeding

Each 150L experimental tank was stocked with 225 all male tilapia fingerlings of 2g average weight. The fish were acclimated for two weeks. Fish were hand fed twice a day at 8% of their body weight.

Feed Intake, Fecal Matter, Nutrient Digestibility and Growth Performance

Feed intake for the experimental period was calculated as average weight of feed taken per fish. All uneaten feed was accounted for throughout the experiment. Fecal matter was collected in sedimentation tanks covered with an ice jacket for nine days. Feces were collected daily before feeding from each tank using the sedimentation column which was housed in a Styrofoam jacked that contained ice to prevent the breakdown of nutrient in the feces during collection. The samples were transported on ice to the laboratory stored at -20^0C to slow down bacterial decomposition until analyzed. Fecal matter for the estimation of fecal output were oven dried (105ºC) and expressed as gDMkg-1 of ingested feed.

The dry matter, lipid, and protein digestibility of each diet was determined by comparing the quantity of nutrients consumed with what remained in the feces at the end of the digestive process. For each treatment, the proximate composition of the diet and the feces were determined at the Faculty of Renewable natural Resources at KNUST following methods described in AOAC (2005). Digestibility was expressed as percentage (%) of the absorbed nutrients divided by the nutrients in the feed according to Jobling (1994).

$$Digestibility\ (\%) = \frac{(Nutrient\ in\ feed - Nutrient\ in\ faeces)}{Nutrient\ in\ feed} * 100$$

Growth performance was estimated based weight gain at the end of a three week growth trial and specific growth rate calculated as:

$$SGR\ (\%/day) = \frac{(LnFW - LnIW)}{D},$$

Where:

IW is the initial weight and FW is the final weight and D is the number of days between weighing.

[1] Tables A1-9 are listed in the "Additional Tables and Figures" section at the end of this report.

Ammonia Excretion Rates
Ammonia excretion rates were determined for each diet. Fish were starved 24 hours to ensure that previously eaten food was removed from their system. Fish in each tank were then fed a single diet of 2% of total biomass. After feeding, the recirculation system was shut down and 15ml water samples were taken from each tank every 3 hours over a 24-hour period for TAN measurements. The total ammonia-nitrogen (TAN) concentrations in water were determined by the use of a YSI 9300 photometer.

Experiment E6
At each fish sampling event during E5, four hapas were randomly selected and five fish from these hapas were harvested for gut content analyses. Zooplankton species and numbers in the gut of fish sampled from the various treatments (n=20) were determined for days 10, 20, and 40. The harvested fish were preserved on ice and transported to the FRNR laboratory where the gut for each fish was removed and preserved in 4% formalin. For identification, the content of the preserved stomach was emptied into a petri dish and 2ml sub samples were taken and observed under a Celestron Digital LCD microscope (X40). Zooplankton species were identified to the genus level using an identification guide (Shiel, 1995).

Statistical and Economic Analyses- *Data Analysis*
Experimental data on growth, survival, FCR and other tested variables were analyzed using general linear models; including linear regressions and Analysis of Variance (ANOVA), and response surface methods implemented in R. Proportional response such as survival was analyzed in some cases with logistic regression. Other one-way ANOVA were conducted using the Graphpad Prism 5 software and Tukey multiple comparison tests at an alpha of 0.05 was used in post hoc analyses.

The experimental results provided input to economic analysis of relative profitability of the feeding strategies. Standard methods for profitability analysis, including enterprise budgets followed Engle and Neira (2005). Feed prices for estimating the cost of production were obtained both from the market and from the feed producer's pricing schedule. Current farm-gate prices of tilapia in Ghana at the time of analysis were obtained from the market. Based on extrapolation of the producer's price schedule, the 25% protein feed, for example, would be priced about 15% less per kg compared to the 30% protein feed. It is possible then to determine the cost savings if pond farmers of tilapia can use the 25% feed for grow-out and achieve an insignificant difference in growth performance compared to the 30% feed.

RESULTS

Experiment E1
The overall survival rate had a negative relationship with stocking densities and varied from 77-85%. The logistic regression showed a significant effect of stocking density on survival ($p < 0.001$), although survival was very variable (Figure A1[2]). Growth as a function of Time showed interaction with Feeding Rate, which made a simple linear or non-linear regression not very useful since feeding rate varied by period and effective feeding rate varied by experimental unit as inferred with the survival data. A smoothed (loess) interpolation of growth showed growth stanzas corresponding to the periods and indicating that the feeding rate adjusted by the time period or interval affected growth (Figure A2). A regression capturing the effect of feeding rate and the number of days of growth was used to generate a two-dimensional contour plot which would serve as a look-up table for hatchery operators using the commercial feed for nursery (Figure 2).

[2] Figures A1-4 are listed in the "Additional Tables and Figures" section at the end of this report.

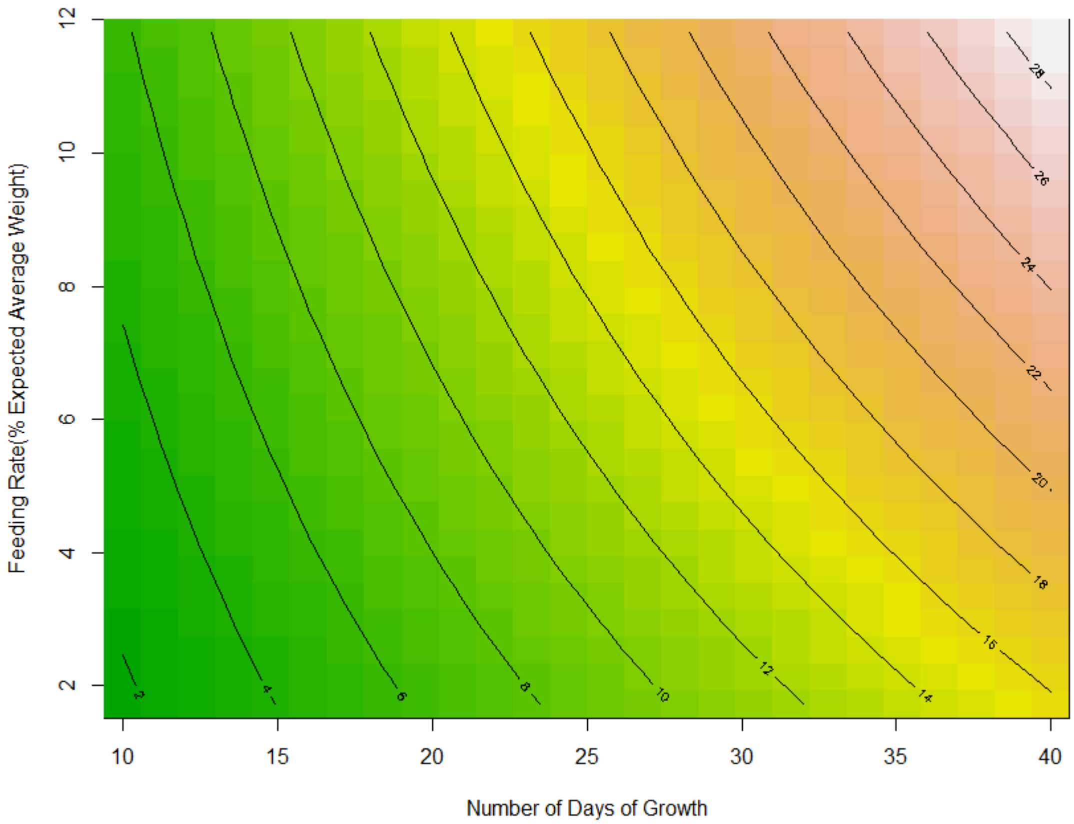

Figure 2.- Predicted weight gain based on the number of days of growth and feeding rate for fry raised in fertilized ponds and fed 48% CP commercial nursery feed at 33.5ºC.

The SGR and feeding rate had a very tight, near-linear, approximately 1:1 relationship, reflecting a consistent low (FCR < 1) across treatments averaging 0.67(±0.02, std. error). This implies that most of the feed fed was utilized for growth, with little or no overfeeding or waste (Figure 3). Overall and approximate Interval FCRs were not predicted by any variables that were measured. Start Density had the highest (but still very low) correlation with FCR, and a linear regression was not significant ($p > 0.05$). A t-test combining the low density treatments against the high density treatment was also not significant (Figure 4). Therefore, while FCR showed a slight trend of potentially deteriorating at high stocking density, the stocking densities in this study may not have been high enough to be of concern. This relationship is worth further investigation with a larger dataset and in follow-up studies using the design of this experiment. It will require additional treatments at higher stocking densities.

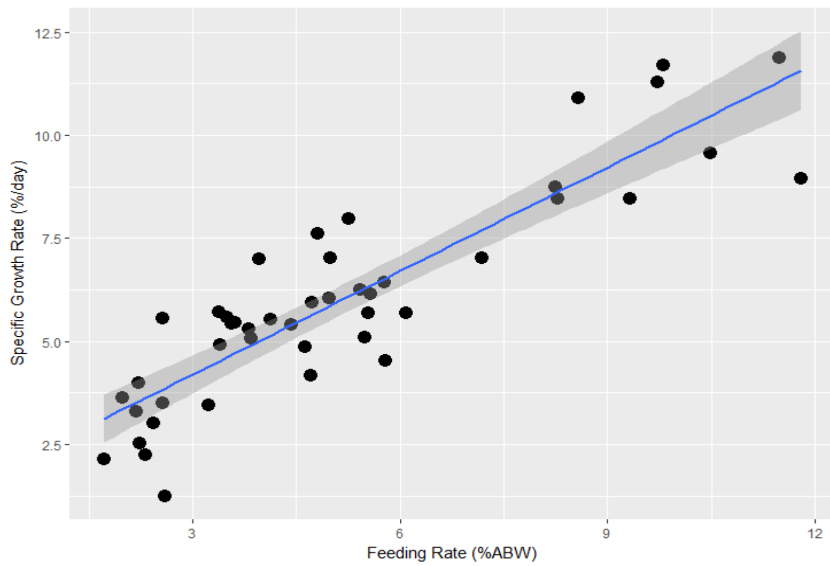

Figure 3.- Relationship between feeding rate and SGR in nursery hapas mounted in fertilized ponds

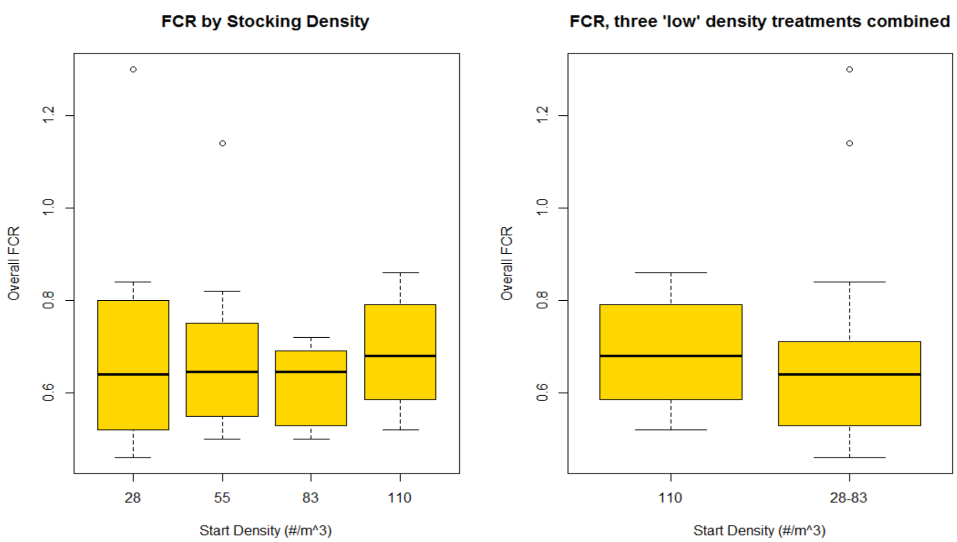

Figure 4. The relationship between FCR and stocking density

Since mortality was not strongly affected by stocking density, the highest treatment was associated with the best profit. An enterprise budget based on a 40-d production cycle to raise fry from 2g to 20g (Table A2) showed a profit margin of > 100% (B/C = 2.08) per fingerling sold. This represented a highly profitable business.

Experiment E4, E5 & E6

Acceptability and Digestibility of Experimental Diets

By the end of the acclimation period, the fish in all treatments readily accepted all the diets used in the experiment and were consuming all the feed fed. Overall feed intake was not significantly different among the different treatments; were 6.63±0.17 (control), 5.76±0.23 (Diet 1), 6.26±0.48 (Diet 2) and 6.67±1.08 (Diet 3). FCR did not differ among the treatments (p>0.05). The control Diet had a slightly higher SGR and weight gain than the experiment diets but not statistically different from the other diets (Table 1).

Table 1.- Growth performance, feed intake and utilisation of juvenile Oreochromis niloticus fed experimental diets for three weeks

Parameter	Control	Diet 1	Diet 2	Diet 3
Initial Weight (g)	1.98±0.39	2.21±0.10	2.23±0.36	1.97±0.29
Final Weight (g)	6.10±0.63	5.74±0.19	6.15±0.70	5.70±0.75
Weight Gain (g)	4.12±0.69	3.52±0.18	3.93±0.67	3.73±0.60
SGR (%day^{-1})	2.84±0.39	2.26±0.13	2.37±0.30	2.38±0.30
Feed Intake (g/fish)	6.63±0.17	5.76±0.23	6.26±0.48	6.67±1.08
Feed Conversion Ratio	1.65±0.32	1.64±0.04	1.61±0.17	1.79±0.12

Each value is the mean ± SD

Fecal matter output ranged from 192.22 1.39 to 231.77 24.15 gDMKg-1 (Figure 5). Fish in the tanks fed the Control and Diet 1 had a lower fecal output; the control diet had a significantly lower ($p< 0.001$) fecal output compared to Diets 2 and 3. There were no significant differences in the dry matter and protein digestibility of fish fed the different diets, however, the lipid digestibility for Diet 3, was significantly lower than that of the other three diets (Figure 6). Mean temperatures and dissolved oxygen levels remained above 27ºC and 6mg/L respectively throughout the study with pH ranging between 5.75 and 7.83 (Table A3). The TAN excretion rates for all diets followed the same trend with the peak rate occurring 6 hours after feeding for all diets and were as follows: 5.48±0.61 mg kg^{-1} hr^{-1} for the Control Diet, 7.39±1.37 mg kg^{-1} hr^{-1} for Diet 1, 6.94±3.42 mg kg^{-1} hr^{-1} for Diet 2 and 5.69±2.26 mg kg^{-1} hr^{-1}.

Growth Trial with Experimental Nursery Feeds

The survival rates for this experiment ranged from 75-92%; there were no significant differences among the treatments (Table 2). There were no significant differences between the mean weights of the fingerling fed the different diets. FCR was 0.55 in the control, 0.59 for Diet 2 and 0.65 in Diet 3. Feed intake was lowest for Diet 3 compared with the other treatments (Table 2). Mean temperatures were above 28ºC and lowest pH recorded was 6.7. Dissolved oxygen levels were higher in the control pond than in the experimental ponds and Secchi depth was highest for the Diet 2 (Table A4).

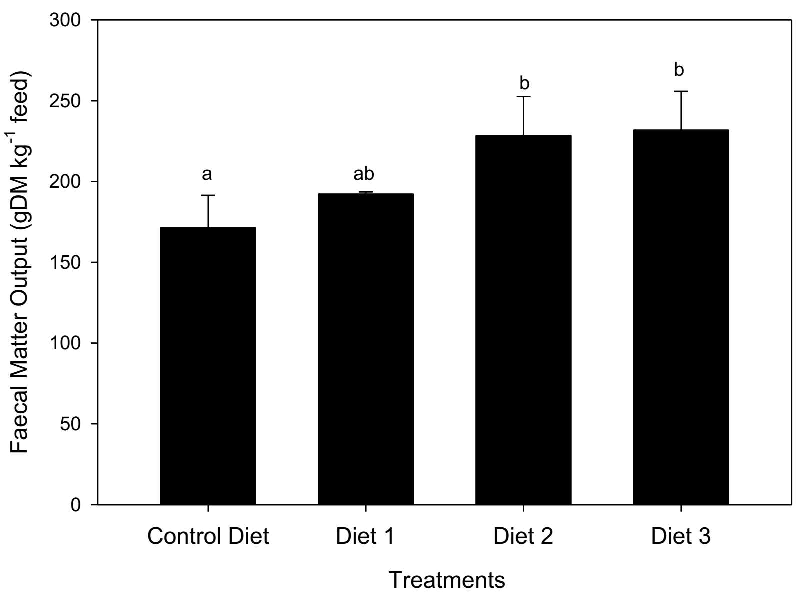

Figure 5.- Faecal matter production (mean ± SD) of Nile tilapia fry fed the different experimental diets.

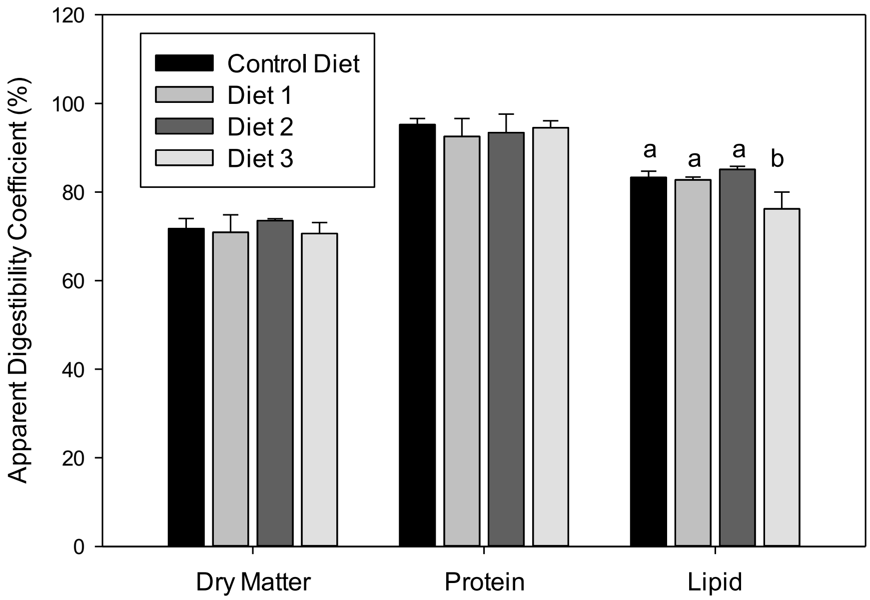

Figure 7. Apparent nutrient digestibility coefficients of the experimental diets and control diets used in this study (treatment means with different letter are significantly different, ANOVA, p<0.05).

Table 2. Survival, growth performance, feed intake and utilization of juvenile Oreochromis niloticus fed experimental diets in hapas in ponds for 40days.

Parameters	**Control**	**Diet 2**	**Diet 3**
Survival (%)	91.75	74.50	87.25
Initial weight (g)	3.95	3.93	3.93
Final weight (g)	20.88	12.91	12.41
Weight gain (g)	16.90	12.84	8.48
Weight gain (%)	427.89	326.34	218.58
FCR	0.55	0.59	0.65
SGR (%/day)	7.06	6.37	5.34
FER	2.85	2.78	1.92
Feed intake (g/fish/day)	0.15	0.12	0.11
Protein efficiency ratio	5.94	5.79	2.39

By day 10, the fingerlings in all treatments were consuming more zooplankton than at the end of the experiment; fish fed the D2 diet generally consumed more zooplankton than the control, and diet 3 (Table A5). The most common zooplankton in the diets for all treatments were the rotifers especially *Brachionus spp* in the first 20 days, the rotifers were still the most encountered species at Day 40 but for diet 3 the ostracods were the most abundant. Frequency of occurrence was highest in Diet 2, followed by the control, with the lowest being Diet 3. Zooplankton numbers were highest at day 20 and lowest at day 40. The most abundant species were the *Trichocerca spp* (Table A5). The phytoplankton encountered was partially digested making it impossible to identify them.

Experiment E2

The detailed statistical and economic analyses presented in this report are based on a concurrent trial at KNUST and a private (ENIN) farm. The trial at KNUST was terminated early due to significant pond volume lost during the dry season overlapping with the experiment. In addition, a high female proportion among the batch of fry led to reproduction occurring in some hapas much earlier than would be anticipated, and before sorting and separating fingerlings. On comparable grow-out periods, both growth (Figure A3) and FCR (Figure A4) were better at the private farm regardless of treatment, pointing to a strong site (ponds) effect. We report a more detailed analysis of data from ENIN farm for a 15-week grow-out period. Overall, the results from the private farm are closer to practical results expected when better management practices are observed. Proximate analysis results closely tracked the manufacturer's label for protein content (Table A5).

Fish weight increased from an initial mean of 59.8 ± 9.5g at the start of the trial to 299.6 ± 10.8g, 277.1 ± 2.0 and 305.4 ± 3.0g for the 25%, 28% 30% protein treatments, respectively (Table 3). There was a significant difference (p = 0.011) in SGR between the 28% CP treatment and the 25% and 30% CP treatments. The 28% treatment had an SGR of 1.21 ± 0.17%/day, 1.27 ± 0.01%/day for the 25% protein and 1.31 ± 0.15%/day for the 30% protein. Mean weight gain also followed the same trend as the specific growth rate with a recorded value of 363.90 ± 60% for the 28% protein, 401.18 ± 18.06% for the 25% protein and 410.90 ± 5.01% for the 30% protein as shown in Table 4.1. The Daily weight gain however, had a different trend from the other growth indices assessed with a significant difference existing only between the 28% and the 30% crude protein treatment. The values for the 25%, 28% and 30% treatments were 1.89± 0.09, 1.74 ± 0.03 and 1.94 ± 0.02, respectively. The FCR averaged 2.06 and protein efficiency ratio average 1.81; both metrics were not significantly different across treatments. Summary of weekly monitored water quality variables are presented in Table A6.

Table 3.- Means ± SD of the growth performance of Nile Tilapia *Oreochromis niloticus*

Variables	**25% CP(n=3)**	**28% CP(n=3)**	**30% CP (n=2)**
Initial Weight (g)	59.78 ± 9.52	59.78 ± 9.52	59.78 ± 9.52
Weight Gain(g)	239.8 ± 10.8^{a}	217.3 ± 2.04^{b}	245.60 ± 3.04^{a}
Weight Gain (%)	401.1 ±18.06^{a}	363.60 ± 3.41^{b}	410.90 ± 5.01^{a}
Final weight (g)	299.58 ±20.32^{a}	277 ±11.56^{b}	305 ± 12.56^{a}
Specific Growth Rate (%/day)	1.27 ± 0.03^{a}	1.21 ± 0.01^{b}	1.29 ± 0.01^{a}
Daily Weight Gain (g)	1.89± 0.09^{ab}	1.74 ± 0.03^{a}	1.94 ± 0.02^{b}
Survival Rate (%)	93.67 ± 3.79^{a}	93.67 ± 5.03^{a}	93.00 ± 0.00^{a}

^{abc}mean values in the same row with different superscripts are significantly different (p<0.05)

The production cost which included cost of feed, labor inputs for pond preparation, pond rental and cost of fingerlings showed that the 28% protein had the highest cost of GH¢12.26 per kg of fish produced. This was followed by the 30% protein which also incurred a production cost per kg of fish at GH¢10.89. The least was the 25% protein which was GH¢9.57. The total cost of production, the revenue generated after sale of fish, and the profit or loss incurred after the trial for both treatments is shown in Table A7. The 28% crude protein treatment also had the highest revenue despite recording the least growth as a result of the proceeds realized from reproduction in the experiment.

Experiment E3

Feed reduction strategies generally did not negatively affect fish growth and nutrient conversion efficiency over the 15 week culture period. Growth was similar among the treatments although fish on control recorded relatively better growth (211.3g) compared to fish that were fed on alternative days (173.3g) (Table 4). Fish in all the treatments had final mean weight that was about four times their initial mean weight which did not differ significantly ($p>0.05$). Other growth performance indices (SGR, FI, SR and DGR) did not show significant differences ($p> 0.05$) among the three treatments. However, the control group recorded the slightly higher performance values. Cumulative feed intake (FI $gfish^{-1}$) over the culture period was significantly higher ($p< 0.05$) for fish fed the control treatment (full ration) but similar for the group on half ration and alternate day full ration (Table 4). Feed conversion efficiency ranged from least efficiency of 1.25 for the control treatment to highest efficiency of 0.86 for the alternate day feeding group. FCRs recorded, however, did not differ among the treatments. Associated water quality data monitored biweekly are presented in Table A8.

Table 4.- Growth performance and feed utilization of *Oreochromis niloticus* fed different feeding strategies culture period of 15 weeks.

Variable	Full Ration	Half Ration	Alternate Day Full Ration
Initial mean weight (g)	35.01 ± 14.81	35.01 ± 14.81	35.01 ± 14.81
Final mean weight (g)	211.29 ± 17.09	185.62 ± 42.53	173.31 ± 20.43
Weight Gain (%)	503.55 ± 48.83	416.37 ± 128.70	380.18 ± 68.12
DGR (g fish-1 day-1)	2.05 ± 0.20	1.75 ± 0.49	1.61 ± 0.24
SGR (%.day-1)	2.09 ± 0.09	1.89 ± 0.27	1.85 ± 0.14
Survival (%)	91.48 ± 6.99	90.71 ± 5.02	91.62 ± 6.76
FCR	1.25 ± 0.23	0.94 ± 0.20	0.86 ± 0.21
FI (g/fish)	219.11 ± 28.98b	134.32 ± 7.38a	116.64 ± 20.69a
Fish yield (kg)	406.43 ± 17.60	350.75 ± 20.55	334.35 ± 18.88
Fish yield (kg/hectare)	3870.81 ± 410.67	3340.47 ± 479.42	3184.28 ± 440.29
Feed given (kg)	418.10 ± 7.42b	255.38 ± 0.00a	222.50 ± 7.39a
Feed given (kg/hectare)	3981.90 ± 173.16 b	2432.14 ± 0.00 a	2119.05 ± 172.40 a

Each value is the mean ± SD of data from three replicates. Mean values with different superscripts in the same row are significantly different at $P < 0.05$. Absence of letters indicates no significant difference between all the treatments. FCR = Feed conversion ratio, FI (g) = Feed intake, SGR (%.day $^{-1}$) = Specific growth rate, DGR (g $fish^{-1}$ day^{-1}) = Daily growth rate.

Economic analysis was based on the experimental and market data and on-farm sale of fish. Variable costs incurred were constant across treatments except feed and labor cost that varied with feeding strategy. Feed cost was significantly ($p < 0.05$) higher in full ration feeding strategy contributing about 43% to operational cost while half ration and alternative day full ration contributed 31% and 34%, respectively. The second largest cost component was labor. The largest labor expenditure contribution to variable cost was realized in the half ration (36%), followed by full ration (30%) whiles the alternate day full ration strategy contributed 25% to total variable cost. Other costs of producing Nile tilapia in fertilized ponds are relatively the same with no regard to a particular feeding strategy. Tilapia produced under alternate day treatment (GH₵ 5.39/kg) resulted in the lowest breakeven price and the full ration was the highest (GH₵ 7.87/kg) (Table A9). Average breakeven prices for all treatments were below the selling price (GH₵ 10.0/kg) of Nile tilapia (at 250g). The average fish yields recorded were above the breakeven yields for all treatments (Table 4). Revenue accrued from fish sale was similar among the three feeding strategies. Net returns above the total cost was significantly higher ($p < 0.05$) in fish fed on alternate day full ration compared to the half ration and full ration. Alternate day full ration recorded a profit of GH₵ 2269.41 representing about 75% profit returns on total money invested, an amount that exceeds the net returns in the full ration by 53%. The half ration feeding strategy recorded a profit of GH₵ 1,582.04 also representing about 42% net returns on investment while the full ration recorded GH₵ 995.88 which represents about 22% returns on investment. None of the feeding strategy however recorded negative returns on investment.

DISCUSSION AND CONCLUSION

The reduced protein and feeding strategies other than full ration for grow-out of tilapia both showed opportunities for substantial reduction in cost and improvement profitability of small-scale, semi-intensive tilapia farming in Ghana. In particular, considering reduced feed cost, the 25% CP feed was substantially more profitable than the 30% CP feed. Similarly, alternate day feeding at full ration, followed by daily feeding at half ration both were more profitable than the full ration feeding of twice daily at 5-2% ABW (depending on size at stocking).

Any reduction in growth associated with reduced protein is more than compensated by the reduced cost of feed. According to the feed manufacturer, opportunities exist for further feed price reduction through decreased vitamin and other supplements as effective pond fertilization provides the necessary nutrients through the phytoplankton and zooplankton. The 28% feed available on local market is designed primarily as a maintenance feed for cage farmers after fish attain market size and before harvest. It is therefore not surprising that this feed formulation appeared to be worse than the 25% feed formulated specifically for pond grow-out of tilapia. The 25% feed is the predominant feed used in Egypt, for example, (El-Sayed 2017) for tilapia grow-out. Farmers need to be educated to understand that slight growth differences due to higher protein content may not be worth the extra cost of feed if profit is to be maximized. Better understanding will increase the acceptability of reduced protein feeds to farmers and consequently motivate its production by the feed manufacturers at a scale that is beneficial to both feed producers and farmers.

Additionally, the opportunity to reduce cost and increase profitability through reduced feeding is well demonstrated by this study. Alternate day feeding at full ration, followed by daily feeding at half ration both were more profitable than the full ration feeding. Farmers might look at the slight differences in growth observed over the study period while missing the economic efficiency associated with the reduced feed input. Increased education of farmers is necessary to draw attention to profitability as the bottom-line to focus on rather than growth. There is also a need to educate farmers to produce for the appropriate market. For example, premium pricing of larger tilapia by the restaurant sector in Ghana pushes small-scale farmers to target production of larger fish which may not necessarily feasible with the strain of tilapia under cultivation in the country but is nevertheless tempting as more feeding appears to result in better growth.

The nursery trial with commercial feed showed profitability at a high stocking density of approximately 100 fry/m^3, without incurring costly high mortalities. Survival rates were quite high across the experimental stocking densities, ranging from 77% at the highest (110/m^3) to 85% at the lowest (25/m^3). Furthermore, it is possible that stocking density could have been increased further to increase profitability even at the risk of slightly increased mortality. Future studies should consider testing higher stocking densities. Based on the current study, a viable tilapia nursery business model is available in Ghana that should be developed.

The experimental nursery feeds also produced highly promising results, considering relatively lower costs associated with production of these feeds from local ingredients. Compared to the commercial feed, survival rates were good in all three experiments with an average overall rate exceeding 75%. The field experiments with 100/m^3 had survival as high as 92%. Combined with the good FCRs (<1) recorded in the field experiments, it is very likely that the conditions under which the fish were grown in this investigation were suitable for juvenile Nile tilapia. Low FCR could also be attributed to the fingerlings feeding on natural foods and this was confirmed by the gut content analysis that showed that plankton was an important part of the fish diet. The importance of plankton in the diet of fingerling has been previously established (Abdel-Tawwab and El-Marakby, 2004; Gupta et al. 2012). Higher survival rates have been associated with feeding with live foods (Hassan 2011). This is further supported by the fact that higher FCR was recorded in the recirculation tanks. In recirculation tanks with bio-filters, bacteria tend to be a more important microorganism than plankton (Hargreaves 2013). All water quality parameters measured in the tanks were within favorable range for tilapia culture with mean dissolved oxygen levels remaining above 6mg/L.

Although, the experimental Diet 1 had good fecal output and good nutrient digestibility, it did not perform as well as the other two experimental diets. Higher fiber content in diets 2 and 3 could account for the higher fecal load from the two treatments. Ammonia excretion rates peaked six hours after feeding, this means that the last feeding of the day should be time such that phytoplankton are still active and can help with the elimination of the ammonia produced. Based on the ammonia excretion rates, survival rates, digestibility and growth performance Diet 2 was the best experimental Diet. This assessment was confirmed during the third experiment where Diet 2 performed better than Diet 3. In addition to the Diet 2 being more digestible than Diet 3, the fish in that treatment eat more zooplankton to supplement their diets than did those fed Diet 3. The experimental diets especially the Diet 2 compared favorably to the control and, given the lower cost, will result in higher profits than that of the control. Stocking densities used had no significant effect on weight gain; similar results were reported by Brown and Bolivar (2005) and did not negatively affect FCR. This might indicate that optimum stocking density is yet to be reached. Collectively, the experiments and economic analyses conducted under this investigation show numerous opportunities still exist from nursery through grow-out in the practice of tilapia production in Ghana, to reduce feed and feeding cost, increase profitability, and sustainably increase tilapia production in the country.

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ADDITIONAL TABLES AND FIGURES

Table A1.- Weight of formulated diets (g kg-1) and proximate composition (% dry weight basis) of feedstuff used for the formulation of the different experimental diets

TEST DIETS			
Ingredients	**DI**	**D2**	**D3**
Fishmeal	486.00	403.00	324.50
Soybean meal	325.00	425.00	509.80
Fermented copra meal	81.00	74.50	71.50
Wheat bran	68.00	57.80	54.20
Binder (starch)	10.00	10.00	10.00
Palm oil	20.00	20.00	20.00
Vit. Supplements	10.00	10.00	10.00

Ingredients	DM		CP		CL		ASH		NFE	
Fishmeal	90.40	91.00	60.80	61.20	5.30	5.60	12.70	13.0		
Soybean meal	90.39	90.26	44.02	44.77	15.09	17.83	9.03	9.15	22.25	18.51
Wheat bran	88.86	88.44	19.73	19.05	9.74	8.51	5.72	5.86	53.67	55.02
Copra meal	83.91	83.72	24.36	23.71	19.45	19.74	8.78	9.08	31.38	31.19
Fermented Copra meal	79.84	80.25	30.10	32.62	11.24	12.13	14.08	14.24	24.42	21.26

DM: Dry Matter; CP: Crude Protein; CL: Crude Lipid; NFE: Nitrogen Free Extract

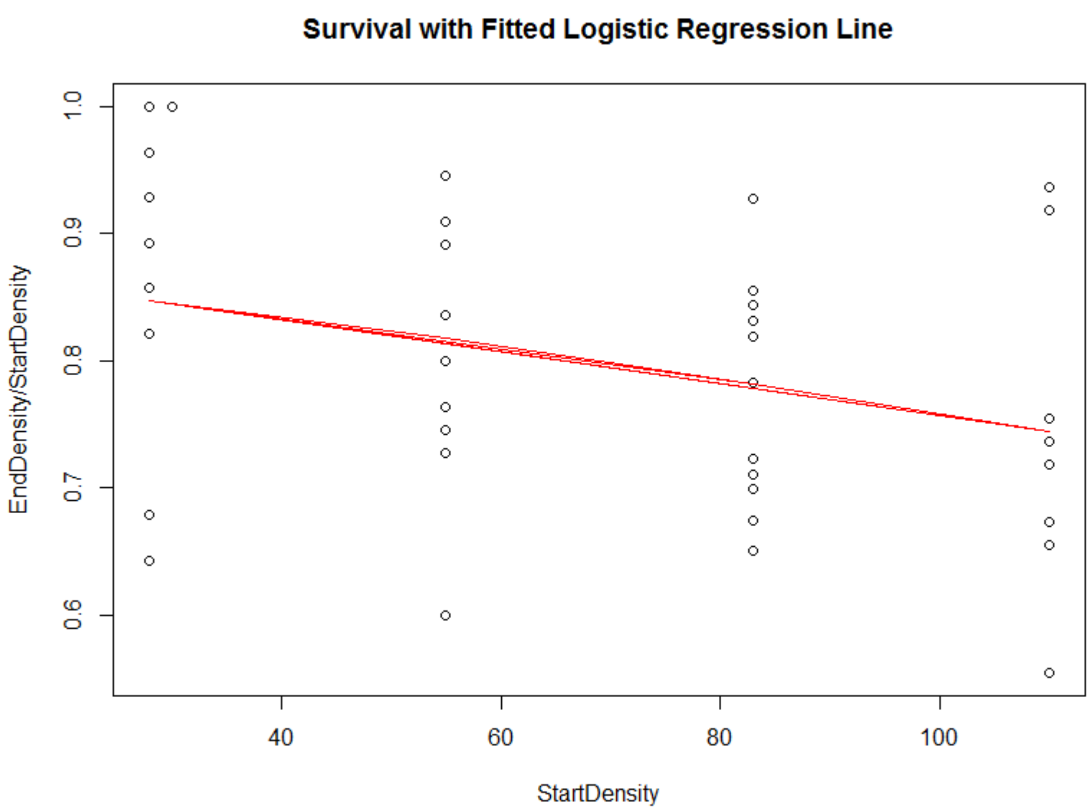
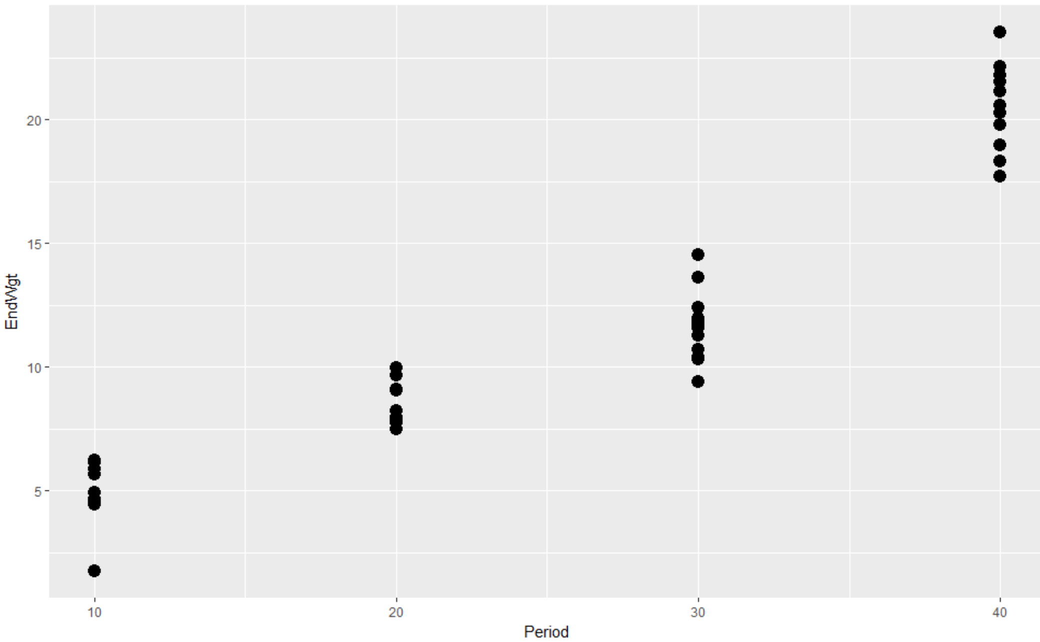


Figure A1.- Survival as a function of FCR in nursery trials. Stocking density in hapas is in number/m^3.8



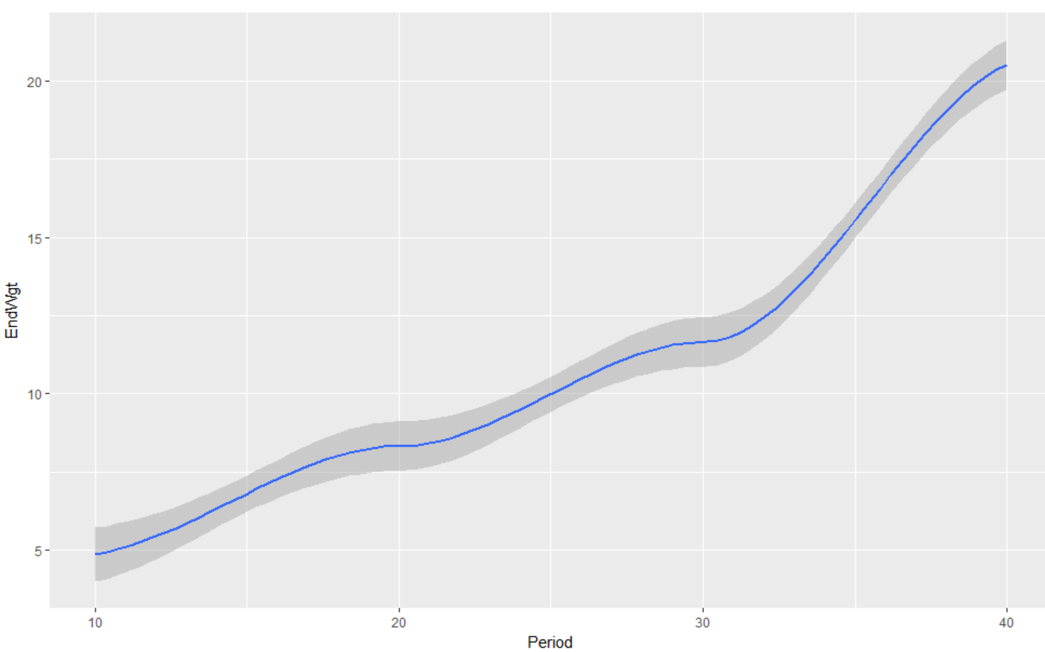


Figure A2.- Weight gain attained in different intervals as influenced by growth rate, **Top**: Scatter plot of end weights (g) against number of days (period) fry/fingerlings are kept in hapas and **Bottom**: A loess smoothing interpolation of end weight (g) by period showing growth stanzas likely caused by differing feeding rates.

Table A2.- Annualized enterprise budget of treatment 110 fish/m^3 for 40-day period at estimated harvest weight of 20g.

ITEM	DESCRIPTION	UNIT	QUANTITY	UNIT COST (¢)	TOTAL (¢)
Gross Receipts					
Tilapia fingerlings	Live (20g) (24% mortality included)	#	1003	1	1003
Total Gross Receipts					1003
Variable Costs					
Tilapia fry	From hatchery(2g)	#	1320	0.17	224.4
Hapa			12	3.08	36.96
Feed	48% CP commercial feed	(kg)	12	10.28	123.36
Fuel		Liter	12	0.95	11.40
Liming		kg	12	2.08	24.96
Labour				3.02	36.24
Fertilizer	MAP & Urea	kg	12	0.1	1.2
Total Variable Cost					458.46
Fixed Cost					
Pond Rent		1m^3		2.08	24.96
Total Cost					482.36
Cost per fingerling		¢/#		0.28	0.28
Fingerling sales		#/¢	0.80	802.56	802.56
Profitability					
Total Revenue					802.56
Gross profit		¢			319.08
Profit Margin					1.08
Profit per fingerling		¢/#			0.52
Breakeven price/fish					
Above TVC		¢/#			0.46
Above TC		¢/#			0.48
Break-even production					
Above TVC		¢/#			458.46
Above TC		¢/#			482.36
Benefit/Cost Ratio		(%)			2.08

Table A3.- Physicochemical variables (mean ± SD) measured throughout the short-term growth trial with experimental feed (n=3 for each diet)

Parameters	Diet 1	Diet 2	Diet 3	Control
DO	6.18 ± 0.52	6.51 ± 0.36	6.76±0.42	6.51±0.48
pH	5.70-7.68	5.76-7.73	5.79-7.83	5.58-7.74
% DO	86.21±4.69	88.26±1.30	84.68±1.98	88.33±1.87
Temperature	28.09±0.63	28.01±0.60	27.98±0.68	28.08±0.64

Table A4.- Physicochemical parameters in field experiments using the experimental; experimental period was 40 days

Parameters	Control	Diet 2	Diet 3
DO (mg/l)	5.07±2.45	3.85±2.08	3.70±1.90
pH	7.19-9.28	6.75-8.63	6.86-8.97
Temperature(°C)	28.43±0.73	28.40±1.11	28.39±0.070
Secchi depth(cm)	19.47±7.96	34.13±6.90	24.38±11.11
Conductivity(µS/cm)	162.50±26.42	145.8±26.26	133.7±23.14

Table A5. Zooplankton identified in fish gut at days 10, 20 and 40 days after the feeding with three different diets

DIET	GROUP	ZOOPLANKTON SPP	FREQUENCY OF O CCURANCE
1ST SAMPLING – DAY 10			
CONTROL	*Ostracod* *Rotifera* *Copepod* *Rotifera*	*Cypria* sp. *Trichocerca* sp. *Cyclops* sp. *Diaptomus* sp. *Brachionus* sp.	2 12 2 1 19
Diet 2	*Ostracod* *Rotifera*	*Cypridopsis* sp. *Brachionus* sp. *Trichocerca* sp.	9 35 17
Diet 3	*Copepod* *Ostracod* *Rotifera* *Copepod*	*Limnocalanus* sp. *Cypridopsis* sp. *Trichocerca* sp. *Brachionus* sp *Diaptomus* sp.	1 5 8 11 1
2ND SAMPLING – DAY 20			
CONTROL	Rotifera Copepod	*Trichotria sp.* *Trichocerca* sp. *Brachionus* sp. *Cyclops* sp.	1 6 8 2
Diet 2	Rotifera	*Trichocerca* sp. *Brachionus* sp.	9 15
Diet 3	Rotifera	*Trichocerca* sp. *Brachionus* sp.	18 4
3RD SAMPLING – DAY 40			
CONTROL	Cladoceran Rotifera cladoceran rotifer Ostracod	*Daphnia* sp. *Trichocerca* sp. *Cyclocypris* sp. *Asplachna* sp. *Darwinula sp.*	1 8 8 1 1
Diet 2	Rotifera Cladoceran Rotifera Ostracod	*Asplachna* sp. *Trichotria* sp. *Trichocerca sp.* *Brachionus* sp. Worm *Polyphemus* sp. *Rotaria* sp. *Cypridopsis* sp.	3 1 7 5 2 1 2 1
Diet 3	Rotifera Ostracod Rotifera Ostracod Ostracod Rotifera	*Macrochaetus* sp. Worm *Cypridopsis* sp *Trichocerca* sp. *Asplachna* sp. *Limnocythere* sp. *Darwinula* sp. *Brachionus* sp	1 1 5 1 1 1 1 1

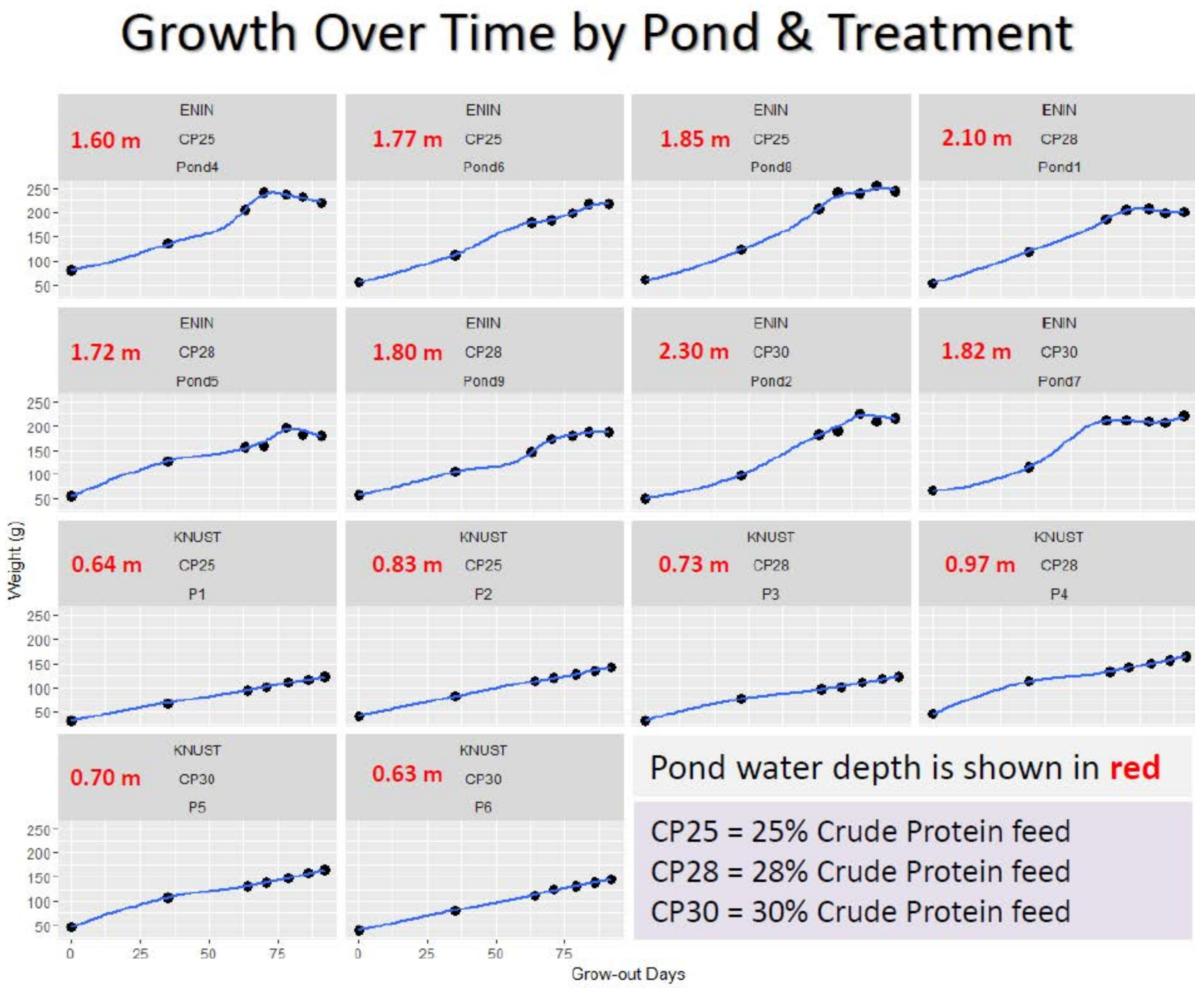


Figure A3.- Fish growth over time in individual ponds at two trial sites.

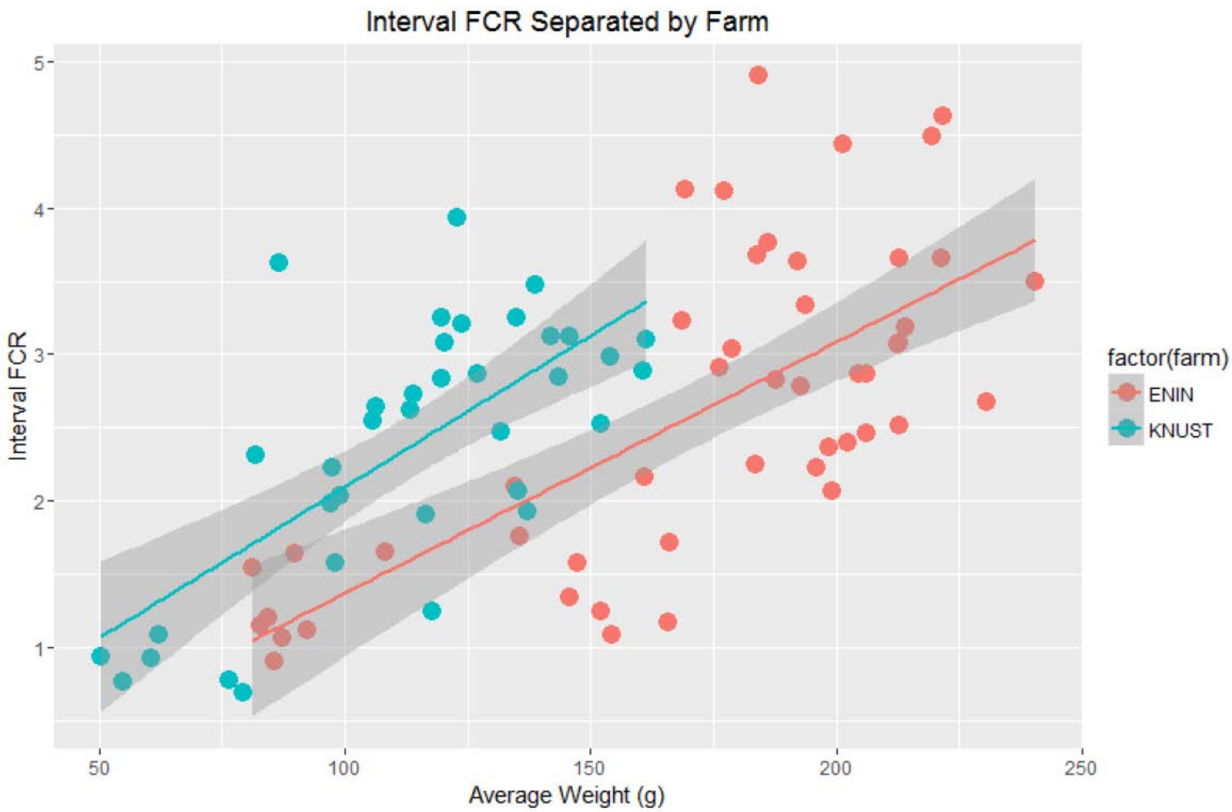


Figure A4.- Interval FCR as a function of fish size (i.e., average weight over the interval) and trial site or farm

Table A5- Proximate analysis results for experimental feeds.

Variable	25% protein	28% protein	30% protein
Protein (%)	25.66	28.56	29.16
Lipid (%)	14.10	16.3	16.90
Fiber (%)	6.00	5.80	5.37
Ash (%)	9.85	10.52	10.55
Moisture (%)	8.55	9.15	9.17

Table A6.- Summary of weekly monitoring of physico-chemical variables of ponds in differing protein content studies

VARIABLE	25% CP	28% CP	30% CP
Temperature (°C)	28.89 ± 1.20	29.31 ± 2.26	28.82 ± 1.88
Dissolved Oxygen (mg/l)	5.28 ± 1.08	4.96 ± 1.43	4.38 ± 1.01
pH (range)	6.13- 6.91	6.40-7.10	6.15-6.80
Conductivity (µS/cm)	23.00 ± 9.96[a]	39.38 ± 11.38[b]	14.25 ± 12.21[a]
Total Dissolved Solids (mg/l)	13.75 ± 10.22[a]	19.63 ± 5.83[b]	6.88 ± 6.49[a]
Secchi Depth (cm)	14.43 ± 2.92[a]	14.81 ± 3.16[a]	15.55 ± 4.40[a]
Chlorophyll-a (µg/L)	495 ± 41.74[a]	427.40 ± 18.26[b]	440.60±13.19[a]
Ammonia (ppm)	0.09 ± 0.05[a]	0.15 ± 0.11[a]	0.14 ± 0.08[a]

[ab]Mean values in the same row with different superscripts are significantly different (P<0.05)

Table A7.- Fish production and economic efficiency of experimental diets per hectare

Variables	**25% CP**	**28% CP**	**30% CP**
Total Production Cost (GHC)	35,017.73	42,966.01	46,479.96
Fish Yield (Kg)			
Table Size (~ 300g)	3709.20 ± 12.35	3503.97± 35.37	4260.42 ± 35.76
Reproduction (kg)	749.26 ± 12.16	1729.20 ± 4.86	1317.24 ± 24.32
Total yield	4458.46 ± 24.51	5233.16 ± 40.23	5577. 66 ± 60.08
Revenue (GH¢)	41,654.30	45,403.05	50,562.50
Profit (GH¢)	6636.57	2437.04	4083.33

Table A8.- Mean values of biweekly monitored physico-chemical variables in the different treatments over the 15 week trial period for ponds under different feeding strategies.

	Feeding Strategies		
Variable	Full Ration (n=3)	Half Ration (n=3)	Alternate Day Full Ration (n=3)
Temperature (°C)	27.54 ± 1.03	27.71 ± 1.23	27.52 ± 0.96
DO (mg/L)	2.91 ± 2.04	2.45 ± 1.63	2.65 ± 1.73
pH (range)	6.80 ± 0.34	6.72 ± 0.34	6.77 ± 0.33
NH_3 (mg/L)	0 ± 0.00	0 ± 0.00	0 ± 0.00
Secchi Depth (cm)	15.49 ± 1.91	16.40 ± 2.22	16.61 ± 4.10
Chlorophyll-a (µg/L)	806.40 ± 489.50	817.32 ± 547.46	897.54 ± 604.17
Nitrite (mg/L)	0.03 ± 0.07	0.04 ± 0.07	0.02 ± 0.04
Phosphate (mg/L)	12.48 ± 16.69	13.19 ± 10.46	23.24 ± 22.04
Water depth (m)	0.97 ± 0.11	0.82 ± 0.08	1.01 ± 0.18
Pond depth (m)	1.37 ± 0.06	1.22 ± 0.06	1.43 ± 0.10

Each value is the mean ± SD of data from three replicates. Mean values with different superscripts in the same row are significantly different at $P < 0.05$. Absence of letters indicates no significant difference between all the treatments. DO – Dissolved Oxygen. Data represents 7 weeks of measurements averaged over 3 replicates per treatment.

Table A9.- Economic analyses of *O. niloticus* fed at different feeding strategies.

Parameters	**Unit**	**Full Ration**	**Half Ration**	**Alternate Day Full Ration**
Yield	kg	548.63 ± 19.40	535.63 ± 25.62	529.89 ± 24.42
Gross revenue	GH₵	5,486.25 ±194.02	5,356.25 ± 256.24	5,298.9 ± 244.23
Total Variable cost (TVC)	GH₵	4,316.54	3,600.38	2,855.65
Fixed costs (FC)	GH₵	173.83	173.83	173.83
Total costs (TC)	GH₵	4,490.37	3,774.21	3,029.49
Breakeven				
Yield above TC	GH₵	449.04	377.42	302.95
price above TC	GH₵	8.18	7.05	5.72
price above TVC	GH₵	7.87	6.72	5.39
Net return				
above TVC	GH₵	1,169.71	1,755.87	2,443.25
above TC	GH₵	995.88	1,582.04	2,269.41
Per Pond	GH₵	331.96	527.35	756.47
Economic Efficiency	%	22.18	41.92	74.91

A Comparison of Monoculture and Polyculture of Tilapia with Carps for Pond Production Systems in Nepal

Production System Design and Best Management Alternatives/Experiment/16BMA03UM

Narayan P. Pandit[1], Madhav K. Shrestha[1], Rama N. Mishra[2], Suresh K. Wagle[3], and James S. Diana[4]

[1]*Agriculture and Forestry University, Nepal*
[2]*Directorate of Fisheries Development, Kathmandu, Nepal*
[3]*Fisheries Research Division, Godawari, Nepal*
[4]*University of Michigan, USA*

ABSTRACT

Carp polyculture is a well-established aquaculture system in Nepal but improving productivity of this aquaculture system as well as the introduction of new aquaculture systems is a major concern. Two trials were conducted to demonstrate the value of Nile tilapia and sahar in polyculture ponds, and a culture system with only monosex tilapia. The on-station trial was conducted at the Aquaculture farm of AFU, Chitwan, Nepal in 12 earthen ponds of 150 m^2 for 185 days (1 June to 3 December 2017). The on-farm trial was conducted in 12 earthen ponds of 200 m^2 in a private farm of Kathar, Chitwan, Nepal for 150 days (17 July to 15 December 2017) to demonstrate the culture potential of sahar and tilapia to farmers. Both trials were conducted in a completely random design with four treatments in triplicate: a) Existing carp polyculture (10,000/ha) + mixed-sex tilapia (3,000/ha) + sahar (1,000/ha) (T_1); b) Existing carp polyculture + monosex tilapia (3,000/ha) (T_2); c) Monosex tilapia at 10,000/ha with fertilization only (T_3); and d) Monosex tilapia at 20,000/ha with fertilization and feeding (T_4). Silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) were stocked in all ponds at the ratio of 3.5:1:2.5:0.5:1.5:1.The mean stocking size of silver carp, bighead carp, common carp, grass carp, rohu and mrigal in T_1 and T_2 in on-station trial was 3.2 and 3.3, 0.6 and 0.6, 13.6 and 12.5, 5.9 and 6.2, 21.7 and 20.6,11.1 and 11.9 g, respectively. The mean stocking size of Nile tilapia (*Oreochromis niloticus*) was 1.9, 0.9, 1.2 and 0.9 g, in T_1, T_2, T_3, and T_4, respectively, and of sahar (*Tor putitora*) was 2.1 g. The ponds were fertilized weekly with urea and di-ammonium phosphate at 4 g N and 1 g P m^{-2} day^{-1}. Fish were fed once daily with commercial pelleted feed (26% CP) at 2% body weight. At harvest, the combined net fish yield was significantly higher ($p<0.05$) in T_4 (3.77 ± 0.23 $t\cdot ha^{-1}\cdot crop^{-1}$) compared to T_3 (1.03 ± 0.14 $t\cdot ha^{-1}\cdot crop^{-1}$); whereas, there was no significant difference ($p>0.05$) among T_1 (2.82 ± 0.23 $t\cdot ha^{-1}\cdot crop^{-1}$), T_2 (3.20 ± 0.17 $t\cdot ha^{-1}\cdot crop^{-1}$) and T_4 (3.77 ± 0.23 $t\cdot ha^{-1}\cdot crop^{-1}$). The mean harvest size, daily weight gain, GFY, and NFY of monosex Nile tilapia in T_4 were significantly higher than in T_3 ($p<0.05$).There was no significant difference in average temperature, total alkalinity, total ammonium nitrogen and chlorophyll-a among treatments during the experimental period ($p>0.05$); however, dissolved oxygen was significantly higher in T_4 than other treatments ($p<0.05$). The gross profit margin was significantly higher in T_4 (4418.5 ± 302.9 USD/ha) compared to T_3 (1666.1 ± 341.3 USD/ha) without any significant difference between T_1 and T_2 ($p<0.05$).

In the on-farm trial, mean stocking size of silver carp, bighead carp, common carp, grass carp, rohu, and mrigal in T_1 and T_2 was 44.2 and 45.3, 3.0 and 3.0, 9.9 and 9.7, 3.0 and 3.0, 29.8 and 32.9 g, respectively. Mean size of mixed-sex tilapia was 3.5 g in T_1 and all-male tilapia of 6.5, 6.5, and 3.2 g size were stocked in T_2, T_3 and T_4, respectively. Similarly, the mean stocking size of sahar in T_1 was 7.0 g. Feeding, fertilization and other culture practices were similar to the on-station trial. At harvest, the combined net fish yield was significantly lower in T_3 (0.71 ± 0.02 $t\cdot ha^{-1}\cdot crop^{-1}$) compared to other treatments ($p<0.05$); whereas, there was no significant difference among T_1 (2.49 ± 0.24 $t\cdot ha^{-1}\cdot crop^{-1}$),

T_2 (2.57±0.36 t·ha^{-1}·crop^{-1}) and T_4 (2.54±0.06 t·ha^{-1}·crop^{-1}) ($p>0.05$). The mean harvest size, daily weight gain, GFY and NFY of monosex Nile tilapia in T_4 were significantly higher than in T_3 ($p<0.05$). There was no significant difference in average temperature and Secchi disk depth among treatments during the experimental period ($p>0.05$); however, dissolved oxygen was significantly higher in T_3 than other treatments ($p<0.05$). The gross profit margin was significantly lower in T_3 (1036.9±70.7 USD/ha) compared to other treatments; whereas, there was no significant difference among T_1 (2521.4±411.8 USD/ha), T_2 (2483.2±339.1 USD/ha) and T_4 (3115.6±237.5 USD/ha) ($p>0.05$).We conclude that the carp-tilapia-sahar polyculture, carps and monosex tilapia polyculture, and monosex tilapia culture with fertilization and feeding systems are equivalent practices and better than the presently used carp polyculture system to enhance pond productivity, species diversification and economically viable aquaculture.

INTRODUCTION

Total fish production in Nepal was 83,000 metric tons in 2016, with about 60% originating from aquaculture. Pond culture is the most popular method of aquaculture, but annual pond yield averages only 4.92 t/ha (DoFD, 2017). Carps are popular warm water fish for culture in Nepal, contributing more than 95% of aquaculture production in the country. Tilapia is a globally prominent species for all types of management intensities. Nile tilapia (*Oreochromis niloticus*) was introduced into Nepal in 1985 (Pantha, 1993), but it remained under government control for more than 10 years (Shrestha and Bhujel, 1999). Since 1996, experiments conducted at Institute of Agriculture and Animal Sciences (IAAS) included polyculture of tilapia and common carp (*Cyprinus carpio*; Shrestha and Bhujel, 1999), mixed-size culture of tilapia (Mandal and Shrestha, 2001), polyculture of grass carp (*Ctenopharyngodon idella*) with tilapia (Pandit et al., 2004), and recently additional polyculture experiments conducted on-station and on-farm (Bhandari et al., 2016). Recruitment control remains a problem, as mixed-sex tilapia is most commonly used for culture. Snakehead (*Channa striata*; Yi et al., 2004) and sahar (*Tor putitora;* Shrestha, 1997) have been evaluated for their ability to control tilapia reproduction by predation on tilapia fry. Tilapia and sahar co-culture was attempted to control excessive recruitment of tilapia and provide an additional species to increase productivity of high-valued indigenous fish (Shrestha et al., 2011). Sahar has been overfished in rivers and lakes, which has resulted in declining populations (Rajbanshi, 2001; Joshi et al., 2002) and listing as an endangered species (IUCN, 2017). While sahar can reduce tilapia fry production, overpopulation of tilapia often occurs even when sahar are present (Paudel et al., 2007; Yadav et al., 2007; Shrestha et al., 2011). Growth of sahar is typically higher in tropical and subtropical ponds than in cages reared in Pokhara lakes, as well as suspended cages in ponds (Bista et al., 2007; Shrestha et al., 2004).

Semi-intensive carp polyculture is an established system in tropical and subtropical regions of Nepal, using fertilized ponds with supplemental feed. The carp species include silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp, grass carp, rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). All 6 species are recommended in certain ratios with a combined density of 10,000 fish/ha, but fingerlings of all species are rarely available when needed for stocking. The typical number of species cultured ranges from four to six. The addition of other proven species (such as tilapia and sahar) with increased stocking density into the existing carp production system could increase productivity up to 57% and net returns by 61% (Shrestha et al., 2012) with no added inputs. Since tilapia consume plankton, they also improve water quality in ponds and in effluents at harvest. Such improvements in water quality, larger economic gain, and production of fish with no further inputs all enhance the sustainability of an aquaculture system environmentally and economically.

In the first phase of the AquaFish Project, we conducted an experiment incorporating tilapia into carp polyculture. The results showed significant increases in yield (29%) and profit margin (81%) when tilapia and sahar were added to carp polyculture (Bhandari et al., 2016). Overall production was still relatively low, about four tons per ha annually. This production is lower than monosex tilapia culture in ponds in Thailand, where we have achieved annual yields of about 5 tons per ha with only fertilizer inputs and up to 20 tons per ha in fed ponds (Diana, 2012). Monoculture of tilapia could possibly outperform polyculture with carps in Nepal, as well, either in terms of total production or in economic returns. It is not possible to directly transfer results on monoculture of tilapia from Thailand to Nepal, given the generally cooler and more seasonal climate in Nepal. Therefore, the purpose of this experiment is to examine monoculture of tilapia along with inclusion of tilapia in polyculture as techniques to best incorporate tilapia into the aquaculture industry in Nepal. Since sahar is an endangered species (IUCN 2017), any success in rearing them could either relieve pressure on wild populations as a food source, or could be used to supplement wild populations by stocking to improving sustainability of aquaculture in Nepal.

The addition of new species to the carp polyculture system and testing of new species under new conditions fit the national aquaculture plans elaborated by government agencies, as well as the *Feed the Future* (FtF) plans for aquaculture improvement. The first FtF research goal is to advance the productivity frontier by both increasing productivity beyond current levels through technology development and extending technology so local production can reach the level of research farms. This study was focused on that goal. Secondly, the national plans for aquaculture and fisheries have goals to improve culture of indigenous fishes and raise yield of ponds from farms to the level of research stations. Again, the present study is in complete alignment with these goals.

This study was intended to expand the technology developed through AquaFish research on carps, tilapia, and sahar production to farmers in order to demonstrate alternative fish production models. In particular, we conducted a new on-farm experiment on monoculture and polyculture systems, using carp with the addition of tilapia and sahar, to determine the most practical system for farm adoption.

OBJECTIVES

The overall goal of this project was to determine if there is a valuable role for tilapia in the aquaculture systems in Nepal. To do this, we had the following objectives:

1. To increase pond productivity through species diversification.
2. To test a carp-tilapia-sahar polyculture and monosex tilapia culture system for outreach potential by private farms.
3. To evaluate the culture potential of sahar and monosex tilapia to farmers.
4. To develop partial enterprise budgets of costs and values of fish crops among treatments.

MATERIALS AND METHODS

Two trials, on-station and on-farm, were conducted simultaneously to evaluate performance of carp-tilapia-sahar polyculture and monosex tilapia culture systems. The on-station trial was conducted at the Aquaculture farm of AFU, Chitwan, Nepal in twelve earthen ponds of 150 m^2 for 185 days (1 June to 3 December 2017). The trial was conducted in a completely randomized design with four treatments in triplicate: a) Existing carp polyculture (10,000/ha) + mixed-sex tilapia (3,000/ha) + sahar (1,000/ha) (T_1); b) Existing carp polyculture and monosex tilapia at 3,000/ha (T_2); c) Monosex tilapia at 10,000/ha with fertilization only (T_3); and d) Monosex tilapia at 20,000/ha with fertilization and feeding (T_4). Silver carp, bighead carp, common carp, grass carp, rohu and mrigal of mean stocking size 3.2 and 3.3, 0.6 and 0.6, 13.6 and 12.5, 5.9 and 6.2, 21.7 and 20.6, 11.1 and 11.9 g, respectively were stocked in T_1 and T_2 at the ratio of 3.5:1:2.5:0.5:1.5:1. Mixed-sex Nile tilapia and sahar of 1.9 and 2.1 g size were added in T_1. Similarly, the stocking size for all-male tilapia in T_2, T_3, and T_4 were 0.9, 1.2, and 0.9 g, respectively. All experimental ponds were completely drained and treated with hydrated lime ($Ca(OH)_2$) at the rate of 450 kg per ha. The ponds were sun dried for 2-3 days then filled with canal water. Ponds were then fertilized at 4 kg N and 1 kg P $m^{-2}day^{-1}$ with di-ammonium phosphate (DAP) (18% N and 46% P_2O_5) and urea (46% N). Fingerlings were stocked one week after pond fertilization. Subsequent fertilizations were done on weekly basis.

Feeding was done with commercial pellet feed (24% CP; Machapuchhre Feed Industry, Kapilvastu, Nepal) at 2% of total carp biomass per day. The proximate composition of feed was 90.0% dry matter, 26.6% crude protein, 8.6% crude fiber, 2.4% ether extract and 5.4% total ash. Feeding was done once in the morning between 0900 and 1000. The quantity of feed was adjusted monthly based on fish sampling. Fertilization with DAP and Urea was done fortnightly in all treatments. Sampling of fish was done monthly from each pond starting 30 days after stocking. During sampling about 10% of the stocked population of each species was weighed to calculate feed quantity for next month, assuming 100% survival. For final harvest, all ponds were drained by pumping and all fish were harvested and weighed.

Weekly and biweekly measurements of water quality parameters were conducted at 0600–0800 h starting from 1 June 2017. Water temperature, dissolved oxygen (DO), pH, and Secchi disk depth were measured in situ weekly using a DO meter (Lutron DO-5519), pH meter (Lutron pH-222) and Secchi disk, respectively. Water samples were collected biweekly from each pond using a plastic column sampler and analyzed for total alkalinity, total ammonium nitrogen (TAN), soluble reactive phosphorous (SRP), and chlorophyll a (APHA et al., 1985). Proximate analysis of feed was done using methods provided in AOAC (1980).

Simple economic analysis was done to determine economic returns from each treatment (Shang, 1990). The economic analysis was mainly based on farm gate price for harvested fish and current local market prices for all other inputs in Nepal. Farm gate prices of sahar, tilapia and carps were 600, 250 and 250 NRs kg^{-1}, respectively. Prices for sahar, mixed-sex tilapia, and monosex tilapia fingerlings were 5, 1, and 2 NRs $piece^{-1}$, respectively. Prices for common carp, silver carp, bighead carp, grass carp, rohu and mrigal fingerlings were 5, 1, 0.5, 2, 5 and 3 NRs $piece^{-1}$, respectively. Prices for DAP, urea and feed was 50, 22 and 20 NRs kg^{-1}, respectively. One $US is equivalent to 105.00 NRs.

The on-farm trial was conducted at the Center for Aquaculture Research and Production (CARP; a private farm), Kathar, Chitwan in twelve earthen ponds of 200 m^2 for 150 days (17 July to 15 December 2017) to verify the culture potential of sahar and tilapia to farmers. The trial was conducted in a completely randomized design with four treatments in triplicate: a) Existing carp polyculture (10,000/ha) + mixed-sex tilapia (3,000/ha) + sahar (1,000/ha) (T_1); b) Existing carp polyculture and monosex tilapia at 3,000/ha (T_2); c) Monosex tilapia at 10,000/ha with fertilization only (T_3); and d) Monosex tilapia at 20,000/ha with fertilization and feeding (T_4). Silver carp, bighead carp, common carp, grass carp, rohu and mrigal of mean stocking size 44.2 and 45.3, 3.0 and 3.0, 9.9 and 9.7, 3.0 and 3.0, 29.8 and 32.9 g, respectively were stocked in six ponds of T_1 and T_2 at the ratio of 3.5:1:2.5:0.5:1.5:1. Mixed-sex Nile tilapia of 3.5 g and sahar of 7.0 g size were added in T_1. Similarly, the stocking size of all-male tilapia in T_2, T_3, and T_4 were 6.5, 6.5, and 3.2 g, respectively. Feeding, fertilization and other culture practices were similar to the on-station trial. The method for economic analysis was similar to that of the on-station trial with slight variation in carp fingerlings. Prices for common carp, silver carp, bighead carp, grass carp, rohu, and mrigal fingerlings were 5, 10, 1, 2, 10, and 5 NRs $piece^{-1}$, respectively.

The data were analyzed by one-way ANOVA using SPSS (V 16.0). For significant differences in growth parameters among different treatments, LSD was used to compare the means. For testing different growth and production parameters of carps, a T-test was used. For all analysis alpha was set at 0.05.

RESULTS

Experiment one: on-station trials

The gross and net fish yields for monosex tilapia without feed was significantly lower than monosex tilapia with feed and carp treatments (Table 1). The production of all carps was not significantly different between T_1 and T_2 ($p>0.05$). The production of monosex tilapia in T_4 was significantly higher than in T_3 ($p<0.05$). Similarly, the extrapolated GFY of tilapia in T_2 was significantly higher than T_1 ($p<0.05$). The combined extrapolated GFY of all species excluding and including tilapia recruits was significantly lower in T_3 than other treatments ($p<0.05$). Similarly, the combined extrapolated NFY of all species excluding tilapia recruits was significantly lower in T_3 than T_4 ($p<0.05$). The apparent food conversion ratio (AFCR) was significantly lower in T_4 compared to T_1 and T_2 without any significant differences between T_1 and T_2 (Table 1).

Table 1. Production parameters (mean ± SE) of different treatments. Mean values in a row with the same superscript are not significantly different (α = 0.05).

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Extrapolated GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)				
Carps	2.42 ± 0.20^a	2.58 ± 0.14^a	-	-
Tilapia	0.49 ± 0.04^a	0.72 ± 0.09^b	1.04 ± 0.14^c	3.79 ± 0.12^d
Sahar	0.02±0.00	-	-	-
Combined excluding tilapia recruits	2.93 ± 0.46^b	3.29 ± 0.17^b	1.04 ± 0.14^a	3.79 ± 0.12^b
Combined including tilapia recruits	3.00 ± 0.22^b	3.29 ± 0.17^b	1.04 ± 0.14^a	3.79 ± 0.12^b
Extrapolated NFY ($t \cdot ha^{-1} \cdot crop^{-1}$) excluding tilapia recruits	2.82 ± 0.23^{ab}	3.20 ± 0.17^{ab}	1.03 ± 0.14^a	3.77 ± 0.23^b
AFCR	2.42 ± 0.28^b	2.09 ± 0.14^b	-	1.86 ± 0.07^a

Each carp species showed similar production parameters in all treatments, indicating the addition of tilapia and sahar did not affect overall carp production (Table 2). There were no significant differences in mean harvest weight, total harvest weight, mean daily weight gain (DWG), survival rate, extrapolated GFY, and extrapolated NFY of different carp species among treatments. However, mean harvest size, daily weight gain, GFY and NFY of monosex Nile tilapia in T_4 were significantly higher than in T_3 ($p<0.05$).

Table 2. Growth and production parameters (mean ±SE) in different treatments in on-station trial. Data based on a 150 m^2 pond for 185 days culture period. Mean values in a row with the same superscript are not significantly different (α = 0.05).

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Common Carp				
Mean stocking weight (g)	13.6 ± 1.1^a	12.5 ± 0.4^a	-	-
Total stocking weight (kg)	0.51 ± 0.04^a	0.48 ± 0.02^a	-	-
Mean harvest weight (g)	385.6 ± 46.8^a	397.0 ± 10.4^a	-	-
GFY (kg)	9.6 ± 0.7^a	10.6 ± 1.3^a	-	-
DWG ($g \cdot day^{-1}$)	2.01 ± 0.26^a	2.08 ± 0.05^a	-	-
Survival (%)	67.5 ± 8.9^a	71.1 ± 10.6^a	-	-

Parameter	Treatment			
	T_1	T_2	T_3	T_4
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.64±0.04 [a]	0.71±0.09 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.61±0.04 [a]	0.68±0.09 [a]	-	-
Silver Carp				
Mean stocking weight (g)	3.2±0.2 [a]	3.3±0.2 [a]	-	-
Total stocking weight (kg)	0.17±0.01 [a]	0.18±0.01 [a]	-	-
Mean harvest weight (g)	319.6±15.8 [a]	370.3±13.6 [a]	-	-
GFY (kg)	6.6±0.3 [a]	5.9±1.2 [a]	-	-
DWG ($g \cdot day^{-1}$)	1.71±0.08 [a]	1.08±0.07 [a]	-	-
Survival (%)	39.0±2.3 [a]	29.6±4.9 [a]	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.44±0.02 [a]	0.39±0.08 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.43±0.02 [a]	0.38±0.08 [a]	-	-
Bighead Carp				
Mean stocking weight (g)	0.6±0.0 [a]	0.6±0.0 [a]	-	-
Total stocking weight (kg)	0.01±0.00 [a]	0.01±0.00 [a]	-	-
Mean harvest weight (g)	470.4±39.6 [a]	473.7±47.0 [a]	-	-
GFY (kg)	4.8±0.1 [a]	4.9±0.2 [a]	-	-
DWG ($g \cdot day^{-1}$)	2.54±0.21 [a]	2.56±0.25 [a]	-	-
Survival (%)	68.9±5.9 [a]	71.1±8.9 [a]	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.32±0.00 [a]	0.33±0.01 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.32±0.00 [a]	0.33±0.01 [a]	-	-
Grass Carp				
Mean stocking weight (g)	5.9±0.3 [a]	6.2±0.4 [a]	-	-
Total stocking weight (kg)	0.05±0.00 [a]	0.05±0.00 [a]	-	-
Mean harvest weight (g)	446.0±195.5 [a]	635.9±47.7 [a]	-	-
GFY (kg)	2.5±1.0 [a]	3.1±0.5 [a]	-	-
DWG ($g \cdot day^{-1}$)	2.38±1.06 [a]	3.40±0.26 [a]	-	-
Survival (%)	79.2±30.1 [a]	62.5±12.5 [a]	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.16±0.06 [a]	0.21±0.03 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.16±0.06 [a]	0.20±0.04 [a]	-	-
Rohu				
Mean stocking weight (g)	21.7±1.2 [a]	20.6±0.7 [a]	-	-
Total stocking weight (kg)	0.50±0.03 [a]	0.47±0.02 [a]	-	-
Mean harvest weight (g)	396.3±40.2 [a]	411.3±62.5 [a]	-	-
GFY (kg)	8.5±0.7 [a]	8.9±0.8 [a]	-	-
DWG ($g \cdot day^{-1}$)	2.02±0.22 [a]	2.11±0.34 [a]	-	-
Survival (%)	94.2±5.8 [a]	95.7±9.1 [a]	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.57±0.05 [a]	0.59±0.05 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.53±0.05 [a]	0.56±0.05 [a]	-	-
Mrigal				
Mean stocking weight (g)	11.1±0.3 [a]	11.9±0.4 [a]	-	-
Total stocking weight (kg)	0.17±0.00 [a]	0.18±0.01 [a]	-	-
Mean harvest weight (g)	471.1±45.6 [a]	480.0±42.1 [a]	-	-
GFY (kg)	4.3±0.7 [a]	5.2±0.3 [a]	-	-

Parameter	Treatment			
	T_1	T_2	T_3	T_4
DWG (g·day^{-1})	2.49±0.25^a	2.53±0.23^a	-	-
Survival (%)	60.0±3.0^a	73.3±3.9^a	-	-
GFY (t·ha^{-1}·crop^{-1})	0.29±0.04^a	0.35±0.02^a	-	-
NFY (t·ha^{-1}·crop^{-1})	0.27±0.04^a	0.34±0.02^a	-	-
Tilapia				
Mean stocking weight (g)	1.9±0.1	0.9±0.0	1.2±0.0	0.9±0.1
Total stocking weight (kg)	0.09±0.01	0.04±0.00	0.18±0.00	0.26±0.02
Mean harvest weight (g)	267.1±28.4	356.5±33.6	152.1±29.2^a	281.7±4.8^b
GFY (kg)	7.42±0.59	10.75±1.30	15.63±2.15^a	56.80±1.77^b
DWG (g·day^{-1})	1.43±0.15	1.92±0.18	0.82±0.16^a	1.52±0.03^b
Survival (%)	62.2±2.3	66.7±2.6	70.0±5.1^a	67.2±1.9^a
GFY (t·ha^{-1}·crop^{-1})	0.49±0.04	0.72±0.09	1.04±0.14^a	3.79±0.12^b
NFY (t·ha^{-1}·crop^{-1})	0.49±0.04	0.71±0.09	1.03±0.14^a	3.77±0.12^b
Sahar				
Mean stocking weight (g)	12.1±1.1	-	-	-
Total stocking weight (kg)	0.18±0.02	-	-	-
Mean harvest weight (g)	35.7±1.9	-	-	-
GFY (kg)	0.37±0.02	-	-	-
DWG (g·day^{-1})	0.13±0.01	-	-	-
Survival (%)	68.9±5.9	-	-	-
GFY (t·ha^{-1}·crop^{-1})	0.02±0.00	-	-	-
NFY (t·ha^{-1}·crop^{-1})	0.01±0.00	-	-	-

There was no significant difference in average temperature, total alkalinity, total ammonium nitrogen and chlorophyll-a among treatments during the experimental period (Table 3, $p>0.05$); however, dissolved oxygen was significantly higher in T_4 than other treatments ($p<0.05$). The soluble reactive phosphorous was significantly higher in T_2 and T_3 than T_4.

Table 3. Water quality parameters (mean ±SE with range in parentheses) of different treatments. Mean values in a row with same superscript are not significantly different ($\alpha = 0.05$).

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Water temperature (°C)	28.3±0.2^a (21.2-32.2)	28.2±0.2^a (21.7-31.6)	28.1±0.1^a (21.5-31.6)	28.1±0.2^a (21.5-31.6)
Dissolved oxygen (mg/L)	2.6±0.1^a (0.7-7.1)	2.7±0.3^a (0.7-6.3)	2.6±0.2^a (0.5-5.7)	3.6±0.3^b (0.7-8.3)
pH	7.2 (6.5-7.9)	7.1 (6.1-8.0)	7.2 (6.2-7.9)	7.2 (6.5-8.2)
Total alkalinity (mg/L as $CaCO_3$)	146.4±13.0^a (86.8-178.5)	143.8±2.2^a (107.5-179.4)	145.3±6.7^a (104.1-199.7)	141.0±9.3^a (104.6-180.9)
Soluble reactive phosphorous (mg/L)	0.43±0.00^{ab} (0.05-0.96)	0.46±0.00^b (0.01-1.08)	0.47±0.02^b (0.14-1.63)	0.37±0.03^a (0.06-0.96)
Total ammonium nitrogen (mg/L)	0.44±0.03^a (0.05-1.27)	0.39±0.02^a (0.08-1.20)	0.42±0.01^a (0.04-1.10)	0.33±0.05^a (0.04-0.88)
Chlorophyll-a (mg/m^3)	78.1±27.7^a (15.9-216.6)	73.7±9.7^a (19.0-210.2)	80.8±10.3^a (12.7-206.3)	48.9±4.8^a (15.1-103.1)

The gross margin for monosex tilapia with feed was significantly higher than monosex tilapia without feed, while carp treatments were intermediate in gross margin. The variable costs in all treatments consisted of seed, feed, lime, urea, and DAP (Table 4). Cost of seed was significantly different among treatments ($p<0.05$), whereas cost of feed was not significantly different among fed treatments ($p>0.05$). There was no significant difference in all other variable costs among different treatments ($p>0.05$). Total input cost and total output were significantly lower in T_3 than other treatments. The gross profit margin was significantly higher in T_4 (4418.5±302.9 USD/ha) compared to T_3 (1666.1±341.3 USD/ha) without any significant difference between T_1 and T_2 ($p<0.05$; Table 4).

Table 4. Economic analysis (in USD) for each treatment. Data based on a 150 m^2 pond area and culture period of 150 days. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Variable	Treatment			
	T_1	T_2	T_3	T_4
Seed	$6.3±0.0^{d}$	$4.5±0.0^{b}$	$2.9±0.0^{a}$	$5.7±0.0^{c}$
Feed	$51.8±3.1^{a}$	$51.3±1.0^{a}$	0.0±0.0	$53.9±0.7^{a}$
Lime	$1.3±0.0^{a}$	$1.3±0.0^{a}$	$1.3±0.0^{a}$	$1.3±0.0^{a}$
Urea	$2.9±0.0^{a}$	$2.9±0.0^{a}$	$2.9±0.0^{a}$	$2.9±0.0^{a}$
DAP	$5.1±0.0^{a}$	$5.1±0.0^{a}$	$5.1±0.0^{a}$	$5.1±0.0^{a}$
Total Input	$67.5±3.1^{b}$	$65.2±1.0^{b}$	$12.2±0.0^{a}$	$69.0±0.7^{b}$
Total Output	$106.0±8.2^{b}$	$117.6±6.0^{bc}$	$37.2±5.1^{a}$	$135.2±4.2^{c}$
Gross Margin	$38.5±10.2^{ab}$	$52.4±6.7^{ab}$	$25.0±5.1^{a}$	$66.3±4.5^{b}$
Gross Margin (ha^{-1})	$2569.1±679.9^{ab}$	$3491.9±449.5^{ab}$	$1666.1±341.3^{a}$	$4418.5±302.9^{b}$

Experiment two: on-farm trials

As in the first experiment, the gross and net fish yields in monosex tilapia without feed were significantly lower than monosex tilapia with feed and carp treatments (Table 5). The production of all carps was not significantly different between T_1 and T_2 ($p>0.05$). The production of tilapia in T_4 was significantly higher than T_3 ($p<0.05$). The combined extrapolated GFY of all species excluding and including tilapia recruits was significantly lower in T_3 than other treatments ($p<0.05$). Similarly, the combined extrapolated NFY of all species excluding tilapia recruits was significantly lower in T_3 than other treatments ($p<0.05$). The apparent food conversion (AFCR) was significantly lower in T_4 compared to T_1 and T_2 without any significant differences between T_1 and T_2 (Table 5).

Table 5. Production parameters (mean ± SE) of different treatments. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Extrapolated GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)				
Carps	$2.35±0.25^{a}$	$2.25±0.02^{a}$	-	-
Tilapia	$0.34±0.07^{a}$	$0.60±0.14^{b}$	$0.78±0.23^{b}$	$2.60±0.06^{c}$
Sahar	0.06±0.00	-	-	-
Combined excluding tilapia recruits	$2.75±0.24^{a}$	$2.85±0.37^{a}$	$0.78±0.3^{b}$	$2.60±0.06^{a}$
Combined including tilapia recruits	$2.81±0.25^{a}$	$2.85±0.37^{a}$	$0.78±0.03^{b}$	$2.60±0.06^{a}$
Extrapolated NFY ($t \cdot ha^{-1} \cdot crop^{-1}$) excluding tilapia recruits	$2.49±0.24^{a}$	$2.57±0.36^{a}$	$0.71±0.02^{b}$	$2.54±0.06^{a}$
FCR	$2.23±0.20^{b}$	$2.24±0.10^{b}$	0.00	$1.60±0.13^{a}$

There were no significant differences in mean harvest weight, total harvest weight, mean daily weight gain (DWG), survival rate, extrapolated GFY, and extrapolated NFY of different carp species among treatments (Table 6). However, mean harvest size, daily weight gain, GFY and NFY of monosex Nile tilapia in T_4 were significantly higher than in T_3 ($p<0.05$).

Table 6. Growth and production parameters (mean ±SE) in different treatments in on-station trial. Data based on a 200 m^2 pond for 150 days culture period. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Common Carp				
Mean stocking weight (g)	9.9±1.12 ^{a}	9.7±0.37 ^{a}	-	-
Total stocking weight (kg)	0.50±0.06 ^{a}	0.48±0.02 ^{a}	-	-
Mean harvest weight (g)	310.6±33.4 ^{a}	302.2±44.3 ^{a}	-	-
GFY (kg)	14.3±1.7^b	9.3±0.9 ^{a}	-	-
DWG (g·day^{-1})	2.00±0.22 ^{a}	1.95±0.30 ^{a}	-	-
Survival (%)	92.0±4.2^b	63.3±6.6 ^{a}	-	-
GFY (t·ha^{-1}·crop^{-1})	0.71±0.07 ^{a}	0.47±0.05 ^{a}	-	-
NFY (t·ha^{-1}·crop^{-1})	0.69±0.07 ^{a}	0.44±0.05 ^{a}	-	-
Silver Carp				
Mean stocking weight (g)	44.2±0.73 ^{a}	45.3±2.91 ^{a}	-	-
Total stocking weight (kg)	3.09±0.05 ^{a}	3.17±0.20 ^{a}	-	-
Mean harvest weight (g)	216.5±27.6 ^{a}	262.0±18.8 ^{a}	-	-
GFY (kg)	14.57±2.07 ^{a}	14.40±0.34 ^{a}	-	-
DWG (g·day^{-1})	1.15±0.19 ^{a}	1.44±0.14 ^{a}	-	-
Survival (%)	95.7±1.7^b	79.1±3.9 ^{a}	-	-
GFY (t·ha^{-1}·crop^{-1})	0.73±0.10 ^{a}	0.72±0.02 ^{a}	-	-
NFY (t·ha^{-1}·crop^{-1})	0.57±0.11 ^{a}	0.56±0.02 ^{a}	-	-
Bighead Carp				
Mean stocking weight (g)	3.0±0.14 ^{a}	3.0±0.50 ^{a}	-	-
Total stocking weight (kg)	0.06±0.00 ^{a}	0.06±0.01 ^{a}	-	-
Mean harvest weight (g)	202.3±65.1 ^{a}	164.2±19.5 ^{a}	-	-
GFY (kg)	2.41±0.49 ^{a}	2.37±0.17 ^{a}	-	-
DWG (g·day^{-1})	1.33±0.43 ^{a}	1.07±0.13 ^{a}	-	-
Survival (%)	66.7±14.5 ^{a}	75.0±12.6 ^{a}	-	-
GFY (t·ha^{-1}·crop^{-1})	0.12±0.02 ^{a}	0.12±0.01 ^{a}	-	-
NFY (t·ha^{-1}·crop^{-1})	0.12±0.02 ^{a}	0.12±0.01 ^{a}	-	-
Grass Carp				
Mean stocking weight (g)	3.0±0.33 ^{a}	3.0±0.00 ^{a}	-	-
Total stocking weight (kg)	0.03±0.00 ^{a}	0.03±0.00 ^{a}	-	-
Mean harvest weight (g)	698.2±136.6 ^{a}	658.3±50.0 ^{a}	-	-
GFY (kg)	4.24±0.32 ^{a}	3.89±0.09 ^{a}	-	-
DWG (g·day^{-1})	4.63±0.91 ^{a}	4.37±0.33 ^{a}	-	-
Survival (%)	63.3±6.7 ^{a}	60.0±5.8 ^{a}	-	-
GFY (t·ha^{-1}·crop^{-1})	0.21±0.02 ^{a}	0.19±0.00 ^{a}	-	-
NFY (t·ha^{-1}·crop^{-1})	0.21±0.02	0.19±0.00 ^{a}	-	-

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Rohu				
Mean stocking weight (g)	29.8±1.16 [a]	32.9±1.66 [a]	-	-
Total stocking weight (kg)	0.89±0.03 [a]	0.99±0.05 [a]	-	-
Mean harvest weight (g)	312.9±35.3 [a]	384.5±24.2 [a]	-	-
GFY (kg)	8.72±0.84 [a]	10.87±0.54 [a]	-	-
DWG ($g \cdot day^{-1}$)	1.89±0.23 [a]	2.34±0.17 [a]	-	-
Survival (%)	93.3±1.9 [a]	94.4±2.9 [a]	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.44±0.04 [a]	0.54±0.03 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.39±0.04 [a]	0.49±0.03 [a]	-	-
Mrigal				
Mean stocking weight (g)	17.33±1.17 [a]	17.67±0.33 [a]	-	-
Total stocking weight (kg)	0.35±0.02 [a]	0.35±0.01 [a]	-	-
Mean harvest weight (g)	220.2±30.5 [a]	259.5±31.6 [a]	-	-
GFY (kg)	2.90±0.13 [a]	4.14±0.96 [a]	-	-
DWG ($g \cdot day^{-1}$)	1.35±0.20 [a]	1.61±0.21 [a]	-	-
Survival (%)	68.3±9.3 [a]	78.3±11.7 [a]	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.15±0.01 [a]	0.21±0.05 [a]	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.13±0.01 [a]	0.19±0.05 [a]	-	-
Tilapia				
Mean stocking weight (g)	3.5±0.48	6.5±0.00	6.5±4.48 [a]	3.2±0.11 [a]
Total stocking weight (kg)	0.21±0.03	0.39±0.00	1.31±0.90 [a]	1.28±0.04 [a]
Mean harvest weight (g)	196.1±21.6	279.8±47.5	132.6±7.4 [a]	214.3±5.6 [b]
GFY (kg)	6.76±1.40	11.93±2.77	15.55±0.59 [a]	52.08±1.13 [b]
DWG ($g \cdot day^{-1}$)	1.28±0.14	1.82±0.32	0.84±0.07 [a]	1.41±0.04 [b]
Survival (%)	56.7±8.6	72.8±17.1	59.2±4.9 [a]	60.8±2.0 [a]
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.34±0.07	0.60±0.14	0.78±0.03 [a]	2.60±0.02 [b]
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.33±0.07	0.58±0.14	0.71±0.06 [a]	2.54±0.06 [b]
Sahar				
Mean stocking weight (g)	7.0±0.14	-	-	-
Total stocking weight (kg)	0.14±0.00	-	-	-
Mean harvest weight (g)	69.4±2.9	-	-	-
GFY (kg)	1.18±0.01	-	-	-
DWG ($g \cdot day^{-1}$)	0.42±0.02	-	-	-
Survival (%)	85.0±2.2	-	-	-
GFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.06±0.00	-	-	-
NFY ($t \cdot ha^{-1} \cdot crop^{-1}$)	0.05±0.00	-	-	-

There was no significant difference in average temperature and Secchi disk depth among treatments during the experimental period (Table 7, $p>0.05$); however, dissolved oxygen was significantly higher in T_3 than other treatments ($p<0.05$).

Table 7. Water quality parameters (mean ±SE with range in parentheses) of different treatments. Mean values in a row with same superscript are not significantly different (α = 0.05).

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Water temperature (°C)	29.2±0.1^a (17.6-32.5)	29.2±0.1^a (17.1-32.3)	28.9±0.3^a (17.0-34.2)	28.9±0.1^a (16.6-31.9)
Dissolved oxygen (mg/L)	5.5±0.1^a (3.3-10.0)	5.3±0.3^a (2.6-10.8)	6.6±0.2^b (2.6-14.1)	5.2±0.3^a (1.4-11.2)
pH	7.8 (6.8-8.7)	7.8 (6.7-8.7)	8.3 (7.2-9.8)	8.0 (7.2-9.0)
Secchi disk depth (cm)	27.0±3.0^a (13.3-46.7)	29.0±3.1^a (18.3-48.3)	34.9±2.9^a (20.0±53.3)	37.6±3.2^a (25.0-60.0)

All carp polyculture and tilapia monoculture systems differed in economic performance, with the treatment monosex tilapia with feed being most profitable. The variable costs in all treatments consisted of seed, feed, lime, urea, and DAP (Table 8). Cost of seed was significantly different among treatments ($p<0.05$), whereas cost of feed was not significantly different among treatments ($p>0.05$). There was no significant difference in all other variable costs among different treatments ($p>0.05$). Total input cost and total output were significantly lower in T_3 than other treatments. The gross profit margin was significantly lower in T_3 than other treatments, whereas there was no significant difference in gross profit margin among T_1, T_2 and T_4 (Table 8).

Table 8. Economic analysis (in USD) for each treatment. Data based on a 200 m^2 pond area and culture period of 150 days. Mean values in a row with the same superscript are not significantly different (α = 0.05).

Variable	Treatment			
	T_1	T_2	T_3	T_4
Seed	14.9±0.0^d	14.5±0.0^c	3.8±0.0^a	7.6±0.0^b
Feed	57.2±7.4^b	58.9±2.3^b	0.0±0.0	41.6±3.0^a
Lime	1.7±0.0^a	1.7±0.0^a	1.7±0.0^a	1.7±0.0^a
Urea	3.9±0.0^a	3.9±0.0^a	3.9±0.0^a	3.9±0.0^a
DAP	6.9±0.0^a	6.9±0.0^a	6.9±0.0^a	6.9±0.0^a
Total Input	84.5±7.4^c	85.9±2.3^c	16.3±0.0^a	61.7±3.0^b
Total Output	134.9±11.6^b	135.6±7.8^b	37.0±1.4^a	124.0±2.7^b
Gross Margin	50.4±8.2^b	49.7±6.8^b	20.7±1.4^a	62.3±4.7^b
Gross Margin (ha^{-1})	2521.4±411.8^b	2483.2±339.1^b	1036.9±70.7^a	3115.6±237.5^b

DISCUSSION

This study was carried out to expand the technology developed through AquaFish research on carps, tilapia and sahar production as well as technology of monosex tilapia production to farmers in order to demonstrate alternative fish production models. An on-station and an on-farm experiment on monoculture and polyculture systems, using carp with the addition of tilapia and sahar was conducted simultaneously, to determine the most practical system for farm adoption. In both trials, addition of Nile tilapia and sahar had no adverse effect on growth and production of all carp species, or in pond water quality. This result suggests that tilapia and sahar did not compete for pond resources with any carp species.

In the on-station trial, the daily weight gain of mixed-sex Nile tilapia and sahar were 1.43 and 0.13 g, respectively, which is comparable or slightly higher than in previous experiments. The daily weight gain of Nile tilapia in polyculture was higher than a grass carp-tilapia polyculture system (0.2-0.5 g;

Pandit et al., 2004), carp-tilapia-sahar polyculture system (0.63-0.70 g; Bhandari et al., 2016), tilapia-sahar polyculture system (0.6-0.9 g; Shrestha et al., 2011), and tilapia-sahar polyculture system (1.15 g; Acharya et al., 2007). The quality of feed (low protein; 26.6% CP) may have also contributed to slow growth of sahar. Sundar et al. (1998) reported better growth, survival, and FCR of sahar were achieved from feed with 45.4% crude protein among diets with 21.4% to 50.2% crude protein. In a similar study, Joshi et al. (1989) reported that 35% crude protein was best for growth and feed efficiency of sahar. We used feed with lower crude protein levels (28%) in this experiment and may have limited sahar growth. Good growth rates of all carp species were achieved in all carp treatments. The average growth rate of carp species in all treatments was higher than reported by previous studies in carp polyculture (Rai et al., 2008; Jaiswal, 2010) as well as in our previous on-farm and farmer's field trials (Bhandari et al., 2016).

The combined gross fish yield in carp treatments (5.8-6.5 $t \cdot ha^{-1} \cdot yr^{-1}$) was higher than the national average of carp polyculture (4.9. $t \cdot ha^{-1} \cdot yr^{-1}$) (DoFD, 2017). The hypothesis that addition of tilapia and sahar would increase the yield and profit from polyculture ponds was supported in the on-station trial. However, production and profit of monosex tilapia without feed was quite low. Diana (2012) achieved annual yields of monosex tilapia about 5 tons per ha with only fertilizer inputs. Although the production of monosex tilapia with feed was higher than all carp treatments and monosex tilapia without feed treatment, this was still quite low than reported by Diana (2012). Although the growth rate was satisfactory, the poor production of monosex tilapia in both feed and non-feed systems in the present experiment was associated with poor survival of fish (67-70%, compared to over 90% in other systems).

In the on-farm trial, growth, production, and survival of carps and tilapia were similar to the on-station trial. However, the growth of sahar was higher (0.42 g $fish^{-1}$ day^{-1}) than the on-station trial (0.13 g $fish^{-1}$ day^{-1}). The daily weight gain of sahar in the on-farm trial was still lower than tilapia-sahar polyculture systems (0.3-0.4 g; Shrestha et al., 2011) and growth rates achieved in other culture systems (0.55-0.77 g; Islam, 2002). In the on-farm trial, the combined gross fish yield in carp treatments (6.7-6.9 $t \cdot ha^{-1} \cdot yr^{-1}$) was slightly higher than the on-station trial (5.8-6.5 $t \cdot ha^{-1} \cdot yr^{-1}$) as well as the national average of carp polyculture (4.9 $t \cdot ha^{-1} \cdot yr^{-1}$; DoFD, 2017).

In both trials, the number of tilapia recruits in the carp-tilapia-sahar system was quite low. This is due to piscivorous nature of the stocked sahar. Shrestha et al. (2011) reported there was a significantly lower average recruit number and weight of Nile tilapia in treatments with sahar than in tilapia monoculture. Jaiswal (2010) also showed that the average number and weight of tilapia recruits in treatments with sahar was lower than with tilapia and carp only.

Water quality was not significantly affected by stocking densities of fishes in species combination of carp-tilapia-sahar polyculture in ponds, as water quality parameters did not differ significantly among treatments. Most water quality parameters in both trials were within acceptable ranges for fish culture (Boyd, 1990).

CONCLUSIONS

The results of this study indicates that three of the culture systems (polyculture of carps with mixed sex tilapia and sahar, carps with monosex tilapia, and monosex tilapia with fertilization and feeding) performed similarly and enhanced productivity and income compared to the currently used carp polyculture system in Nepal. Tilapia either in monoculture or in polyculture proved suitable additional species in the aquaculture for Nepal. As carp polyculture is the established culture system, adding species will be easier to adopt by fish farmers. While adoption of monoculture may be more difficult, indications of higher production efficiency and profit will be the first steps in developing that system. Inclusion of sahar in polyculture will also help in controlling tilapia recruitment in mixed sex tilapia culture where monosex fry is not available along with production of sahar, which will help to conserve sahar populations.

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DEVELOPING NEW SYSTEMS FOR PERIPHYTON ENHANCEMENT IN FARMERS' PONDS

Production System Design and Best Management Alternatives/Study/16BMA04UM

Sunila Rai[1], Kamala Gharti[1], Madhav K. Shrestha[1], Rahul Ranjan[1], and James S. Diana[2]

[1]*Agriculture and Forestry University, Nepal*
[2]*University of Michigan, USA*

ABSTRACT

A field trial was carried out to test four locally available substrates for periphyton enhancement in carp-SIS ponds at Majhui in Chitwan and Seri and Nandapur in Nawalparasi district for seven months. Tested substrates were suggested by farmers at workshops done in each district. Altogether 30 farmers, 15 from Sundardeep women's cooperative in Chitwan and 15 from Mishrit cooperative in Nawalparasi participated in the trial. Five treatments: i) control without substrate, ii) split bamboo, iii) whole bamboo, iv) banana midrib, and v) plastic bottle were introduced to farmers in each district. Farmers were divided into five groups in each district with each group having three farmers adopt one treatment. All farmers stocked 6 carp species at 15,000 fish per hectare and SIS at unrecorded densities. Farmers fed fish with rice bran and mustard oil cake at 1.5% BW/d while grass carp was fed with grass and banana leaves at 50% BW/d. Flood hit both districts in August 2017 and affected the trial. The effect of flooding was greater in Chitwan and pond data varied dramatically. Carp survival and production varied from 13 to 40% and 0.96 to 2.83 $t \cdot ha^{-1} \cdot yr^{-1}$, respectively. In Nawalparasi, damage from flooding was less and data were used to determine treatment effects. Combined NFY was 19% higher in ponds using plastic bottles than control ponds, while NFY of SIS was 50% higher in ponds using banana midribs than control, strip bamboo, and plastic bottle treatments. Feed conversion ratio was less than 1.9 in all substrate ponds, indicating substrates have potential for reducing feed cost. FCR was significantly better ($P<0.05$) in split bamboo ponds than control ponds. Banana midrib decayed fast and required 3-4 replacements during a grow-out while treatments with plastic bottles performed better in terms of production and profit. Water quality was monitored monthly while periphyton abundance and biomass was analyzed three times, in the beginning, middle, and end of trial. Water quality was within an acceptable range for carp. Periphyton abundance and biomass did not differ significantly among substrates. Non-adopting farmers (16 males and 26 females) of Chitwan and Nawalparasi were trained on carp-SIS polyculture in periphyton enhanced systems through a one day workshop.

INTRODUCTION

The government of Nepal has recognized that chronic malnutrition is a major problem in the country. About 41% of children younger than 5 years of age are stunted (UNICEF, 2012) and 48% are anemic (MoHP, 2006). With the nutrition problem, there is a need to develop environmentally sustainable and cost-effective food production systems that function year round to provide adequate nutrients and improve household income for poor rural farmers.

Our research activities in Nepal have targeted local women for improvements in household and larger-scale fish pond production. In Nepal, men from poorer rural areas are often forced to seek employment outside the home (often even outside the country), and women are left to maintain the household and care for the family (Bhujel, 2009). As a result, most ponds developed for household aquaculture are managed by women. Carp-SIS polyculture and carp-SIS polyculture with bamboo substrates managed by Sundardeep women's cooperative have been examples.

In Phase I, we tried to enhance pond production by providing bamboo substrates for colonization of periphyton. As periphyton removes nutrients from the water and adds oxygen as it grows, it also cleans water being discharged from ponds and improves environmental performance. Since rohu, catla, and common carp are periphyton feeders (Rai and Yi, 2012), their growth and production are enhanced in ponds with added substrate for periphyton colonization compared to ponds without substrate (Azim et al., 2002; Rai et al., 2008). We recently completed a series of trials in on-station and on-farm experiments (Rai et al., 2016). These experiments showed dramatic increases in net fish yield (27%) and profit (74%) by adding substrates and reducing feeding rates in on-station experiments. For on-farm studies, total fish production and gross margin were 19.3% and 151% higher in the carp+SIS+substrate treatments with 50% feeding than in carp polyculture with 100% feeding. Reduced feeding that is possible when periphyton is enhanced is not only economically more viable but also enhances environmental performance, as the water quality in ponds is generally higher and effluent released on draining for harvest is not as damaging. However, the on-farm work also identified some problems with our periphyton system. We used fixed rafts of bamboo covering about 1% of the pond area as a substrate for periphyton growth, but culturists believe these structures interfered with harvesting of fish, although on the positive side, they may also have provided hiding places for fish to avoid predation by birds, since survival of some carp species was higher in substrate ponds. Further outreach on this system, including testing of alternative periphyton enhancing substrates that minimize disturbance to their operations, is the main objective of this investigation. Some possible methods might include using portable and floating substrates or ones that could be lifted from the water or pond during management activities. The economic value of periphyton enhancement includes the ability to grow fish faster under similar inputs, as well as the ability to reduce inputs of feed and achieve similar growth rates. However, our previous trials included both periphyton enhancement and feed input reduction together. We have not tested reduced feeding without periphyton enhancement, and thus the gain in profit by reduced feeding has been included in the benefit of periphyton enhancement in our studies to date. We need to also separate these two management activities so we can clearly understand the importance of reduced feeding compared to periphyton enhancement in polyculture systems.

OBJECTIVES

Since previous work using bamboo mats resulted in modifications to ponds that gave better production and economic returns but interfered with pond management, we decided to investigate alternative substrate systems. The purpose of this study is to field test alternative periphyton substrates suggested by farmers in a workshop. The alternative systems were tested on-farm using carps and SIS ponds in two locations of Nepal.

MATERIALS AND METHODS

Periphyton Workshop

Two workshops were held one in Sauraha, Chitwan and one in Kawasoti, Nawalparasi district. The objective of the workshop was to identify possible alternatives for periphyton substrates based on farmers' recommendations. In Chitwan, 32 people participated in the workshop, among them, 16 were farmers (3 male, 13 female), 1 was an executive member of Chitwan Fisheries Entrepreneurial Association, 2 were heads of NGOs, 4 were faculty of Agriculture and Forestry University (AFU), 8 were students (3 male, 5 female), and 1 was an intern from the USA (female). Similarly, 27 people participated the workshop in Nawalparasi, among them, 15 were farmers (13 male and 2 female), 2 were executive members of District Fisheries Entrepreneurial Association, Nawalparasi, 2 were heads of NGOs, 1 was a Fisheries Development Officer, 2 were faculty of AFU, 4 were students of AFU, and 1 was an intern from the USA (female). The participant list is given below in Table 1.

Table 1. Participants in the inception workshop from each district

Participants	Chitwan			Nawalparasi		
	Male	Female	Total	Male	Female	Total
Farmer	3	13	16	13	2	15
Fisheries Entrepreneurial Association	1		1	2		2
NGO	2		2	2		2
Fisheries Development Office						1
Faculty	4		4			2
Student	3	5	8	2		4
Intern		1	1		1	1
Total	**13**	**19**	**32**	**19**	**3**	**27**

At the workshops, presentations on importance of periphyton substrates to fish, different types of substrates and their uses were made to give knowledge of periphyton enhanced fish production system to non-adopting participants. Farmers that have been involved in the previous periphyton studies along with other participants from the district were divided into 5 discussion groups and asked to propose better alternatives than bamboo mats for periphyton substrates in ponds. Prior to discussion, participants were instructed to consider criteria for alternatives that must be i) environmentally responsible, ii) locally available, and iii) cost effective. Participants suggested the following substrates after group discussion.

Table 2. Periphyton substrates recommended by participants

.	Types of substrate	Frequency
1	Split bamboo mat	9
2	Whole bamboo	8
3	Bamboo mat along slope of the dike	1
4	Plastic bottle with sand inside	3
5	Jute sac	1
6	Coconut tree leaf	1
7	Banana leaf midrib	1
8	Boulders	1
9	Galvanized insulated net	1

Based on their recommendations and criteria provided, 4 alternatives including split bamboo, whole bamboo, banana midrib, and plastic bottles were selected for field testing.

On-Farm Trial

An on-farm trial was conducted for 230 days in Majhui of Chitwan district and for 210 days in Seri and Nandapur of Nawalparasi district. Fifteen women farmers from Sundardeep women's fish farmers cooperative in Majhui and same number of farmers (12 male and 3 female) from Mishrit cooperative in Seri and Nandapur participated in the trial. Participant selection was done by respective cooperatives. Lists of farmers in Chitwan and Nawalparasi districts are given below in the Tables 3 and 4. Farmers were divided into five groups including 3 farmers in each group based on substrate types they tested i) control without substrate, ii) split bamboo, iii) whole bamboo, iv) banana midrib and v) plastic bottle.

Table 3. List of farmers used in testing various substrate treatments in Chitwan district

Number of Participants	Gender	Cooperative's name	Substrate type
3	Female	Sundardeep women cooperative	No substrate
3	Female	Sundardeep women cooperative	Split bamboo
3	Female	Sundardeep women cooperative	Whole bamboo
3	Female	Sundardeep women cooperative	Banana midrib
3	Female	Sundardeep women cooperative	Plastic bottle

Table 4. List of farmers used in testing various substrate treatments in Nawalparasi district

Number of Participants	Gender	Cooperative's name	Substrate type
2	Male	Mishrit cooperative	No substrate
1	Female	Mishrit cooperative	No substrate
3	Male	Mishrit cooperative	Split bamboo
3	Male	Mishrit cooperative	Whole bamboo
2	Male	Mishrit cooperative	Banana midrib
1	Female	Mishrit cooperative	Plastic bottle
1	Female	Mishrit cooperative	Banana midrib
2	Male	Mishrit cooperative	Plastic bottle

Farmers prepared ponds by liming at 500 kg/ha. After that they fertilized ponds using urea and DAP at 470 g/100 m^2 and 350 g/100 m^2 to stimulate growth of phytoplankton. Prior to stocking, farmers fixed substrates assigned to them in ponds covering 2% of pond surface area. A split bamboo mat was formed by placing bamboo splits one after another over the vertical bamboo splits. The mat was suspended in the water column by using empty plastic bottles (coke, mineral water bottles) tied to upper part of the mat to float the mat while water filled plastic bottles were tied to the bottom part of the mat to give weight to the mat. The number of bamboo splits used in each mat and number of mats per pond was determined by area of bamboo splits. The area of bamboo splits was estimated by measuring length and width of 5 splits and taking their average. On average, 8-10 pieces of bamboo splits were used per mat. Number of bamboo mats per pond varied from 2-19 depending on pond size. Whole bamboo containing side branches were directly kept in the pond without using floats and sinkers. Due to side branches, the bamboo remained suspended in the water column. Area of whole bamboo was estimated by measuring circumference and length of bamboo pieces while area of side branches was not estimated. Number of whole bamboo pieces used per pond varied from 2-19 depending on pond size. Banana midribs were used as rafts due to the buoyancy caused by air space inside the midrib. Area of each midrib, number of midribs in each mat and number of mats per pond was estimated in the same way as bamboo splits. Number of banana midrib rafts per pond varied from 2-17 depending on pond size. For plastic bottle substrates, water filled plastic bottles were tied to a bamboo strip ring using string. Empty plastic bottles were used as floats which were tied at the upper part of each ring. Area estimation process was similar to whole bamboo. Number of bottles per pond varied from 10 to 30 per ring depending on size of bottle because some farmers used mineral water bottles which are relatively small in size while others used large sized Coke and Fanta bottles. Number of rings containing bottles per pond varied from 2 to 6.

Farmers stocked silver carp (*Hypophthalmichthys molitrix,* 0.7±0.1 g), bighead carp (*Aristichthys nobilis,* 25.6±4.0 g), grass carp (*Ctenopharyngodon idella,* 4.5±0.4 g), common carp (*Cyprinus carpio*, 0.1±0.0 g), rohu (*Labeo rohita,* 4.3±0.3 g), mrigal (*Cirrhinus mrigala*, 4.5±0.3 g) at the rate of 15,000/ha. Stocking was started on 10 April and completed on 12 April 2017 in both districts. They fed dough (overall crude protein=24%, crude fat=6%, crude fiber=9%) of rice bran and mustard oil cake mixed at 1:1 to carp at 1.5% of carp biomass per day as defined in the protocol (full feeding was 3% of carp biomass). Farmers also fed grass carp with banana leaves and grass at 50% of body weight per day. Monthly fish growth samplings were done to adjust the feed ration. Farmers in Chitwan stocked SIS (Dedhuwa *Esomus danricus* and Pothi *Puntius sophore*) by allowing canal water to enter the pond after the monsoon started whereas farmers in Nawalparasi collected SIS from nearby canals for stocking. Final harvest was started on 10 November and completed on 15 November 2017 in Nawalparasi and started on 30 November and completed on 3 December 2017 in Chitwan. Final harvesting was done by draining the pond as much as possible using pumps. Fish were counted and weighed in batches by species to get the final harvest weight and number. After taking weight, fish were released back to a pond for future use. Tharu people consume and sell many fish during "Maghi," their biggest festival which falls on January 15. A record book was given to each farmer to record the number and weight of fish that were consumed, sold, or died. Fish production includes count and weight of those consumed, sold or died along with final harvest. Farmers did not sell SIS because they consumed all at home.

Gross margin analysis
Gross income from fish sales was calculated from total production, assuming all carp and SIS were sold. Selling price of carp was NRs 270/kg (105 NRs = $1 US) in Chitwan and NRs 300/kg in Nawalparasi, and that of SIS was estimated NRs. 200 in both districts. Variable costs of carp seed was estimated NRs 3.25/piece, lime NRs 18/kg, urea NRs 20/kg, DAP NRs 50/kg and feed NRs 32.5/kg. SIS was procured free of cost by farm labor. Gross return was calculated assuming all products sold at farm gate prices.

Water quality monitoring and periphyton analysis
Temperature, DO, pH and Sechhi disk visibility of ponds were monitored in situ monthly. Periphyton samples from four different types of substrate were taken from the field and analyzed three times, in the beginning, middle, and end of the trial at the laboratory of the Fisheries Program in AFU. Periphyton genera were identified and their abundance estimated for each substrate. Periphyton attached to a substrate was scraped with a scalpel from a random 1 cm^2 area of the substrate for periphyton biomass analysis. Dry matter, ash free dry matter, and ash content were determined following APHA (1980).
A one-way analysis of variance (ANOVA) was used to compare periphyton abundance and biomass (dry matter, ash, and ash-free dry matter) among treatments. ANOVA was also used to test differences in fish production and gross return among treatments. Alpha was set at 0.05 for all comparisons. All means are reported with ± 1 standard error.

RESULTS

Water quality was within an acceptable range in all treatment ponds indicating substrate did not affect water quality adversely. Water quality varied slightly between Chitwan and Nawalparasi districts and between treatments within the district. Mean temperature was slightly lower in all treatments in Chitwan than Nawalparasi. Similarly, mean transparency was lower in all treatments in Nawalparasi than Chitwan.

Table 5. Water quality in ponds in each treatment in Chitwan and Nawalparasi districts

	Control	Split Bamboo	Whole Bamboo	Banana Stem	Bottle
			Chitwan		
Temperature	28.9±0.5	28.8±0.4	28.5±0.6	28.3±0.4	28.8±0.6
Transparency	32±6	31±5	29±4	32±4	32±6
DO	4.5±0.8	4.3±0.7	4.1±0.4	4.1±0.8	4.0±1.0
pH	8.5±0.2	8.4±0.2	8.3±0.2	8.3±0.3	8.1±0.3
			Nawalparasi		
Temperature	29.3±0.3	29.8±0.7	30.0±0.8	30.1±0.5	30.1±0.5
Transparency	24±3	25±4	25±3	22±3	23±3
DO	3.9±0.7	4.6±0.8	4.3±0.8	4.5±0.5	4.3±0.6
pH	8.0±0.2	8.2±0.2	8.0±0.2	7.3±0.2	8.1±0.2

There were no significant differences in periphyton abundance and biomass among substrate treatments. Periphyton biomass was determined in terms of dry matter, ash and ash-free dry matter.

Table 6. Abundance (no./cm^2) of periphyton genera in each substrate treatment in Chitwan

Group	Treatment			
	Whole Bamboo	Bottle	Split Bamboo	Banana Stem
Coscinodiscus	4306±1002^{b}	3056±278^{ab}	2083±241^{b}	2083±241^{b}
Cyclotella	9306±773^{a}	3889±501^{b}	5556±1565^{ab}	5833±1339^{ab}
Diatoma	7778±501^{a}	3889±2003^{a}	3750±417^{a}	5833±1502^{a}
Fragillaria	556±278^{a}	0±0^{a}	0±0^{a}	417±417^{a}
Gomphonema	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Melosira	556±556^{a}	417±417^{a}	0±0^{a}	0±0^{a}
Navicula	7778±1528^{a}	4722±1806^{a}	2778±1137^{a}	5694±1389^{a}
Nitzschia	2778±845^{ab}	972±501^{b}	8333±3127^{b}	1111±1111^{b}
Surirella	278±278^{a}	972±972^{a}	972±972^{a}	417±417^{a}
Synedra	6250±1049^{a}	4583±2441^{a}	4028±1187^{a}	5417±241^{a}
Bacillariophyceae	39583±4455^{a}	22500±7051^{a}	27500±3146^{a}	26806±4654^{a}
Actinastrum	0±0^{a}	972±972^{a}	1528±1528^{a}	3056±1547^{a}
Ankistrodesmus	11944±1602^{a}	5417±2295^{b}	8194±972^{ab}	8472±1806^{ab}
Chlamydomonas	1250±636^{a}	1111±1111^{a}	0±0^{a}	0±0^{a}
Characium	0±0^{b}	0±0^{b}	4444±139^{a}	1667±1667^{b}
Chlorella	12500±5838^{a}	4167±1049^{a}	7361±1002^{a}	11250±2320^{a}
Chodatella	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Closterium	1667±1667^{a}	0±0^{a}	0±0^{a}	2778±1690^{a}
Coelastrum	0±0^{a}	1667±1667^{a}	556±556^{a}	0±0^{a}
Cosmarium	3472±1837^{a}	833±636^{a}	139±139^{a}	556±556^{a}
Crucigenia	4306±3319^{a}	3194±2235^{a}	4167±1049^{a}	2500±2097^{a}
Gonatozygon	2361±1773^{a}	1111±1111^{a}	833±833^{a}	0±0^{a}
Mougeotia	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}

Group	Treatment			
	Whole Bamboo	Bottle	Split Bamboo	Banana Stem
Oocystis	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Pediastrum	10000±1909[a]	4306±2183[a]	8333±1925[a]	9583±636[a]
Scenedesmus	4167±3368[a]	2222±1325[a]	6806±1325[a]	7500±2097[a]
Selenastrum	556±556[a]	0±0[a]	694±694[a]	694±367[a]
Sphaerocystis	417±417[a]	0±0[a]	0±0[a]	0±0[a]
Staurastrum	5833±1049[a]	2917±1463[a]	4722±1137[a]	4167±1463[a]
Tetreedron	556±556[a]	0±0[a]	417±241[a]	833±833[a]
Tetraspora	0±0[a]	0±0[a]	1250±722[a]	417±417[a]
Treubaria	556±556[a]	972±501[a]	0±0[a]	139±139[a]
Ulothrix	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Volvox	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Chlorophyceae	**59583±13283[a]**	**28889±12108[a]**	**49444±5195[a]**	**53611±6729[a]**
Anabaena	5972±2639[a]	3333±1925[a]	6806±1187[a]	4167±722[a]
Aphanocapsa	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Chroococcus	2639±1565[a]	2083±1339[a]	1528±139[a]	1389±735[a]
Gloeocapsa	2500±2500[a]	0±0[a]	0±0[a]	0±0[a]
Gomphosphaeria	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Merismopedia	12500±1273[a]	5972±2850[ab]	4306±367[b]	6806±2434[ab]
Microcystis	0±0[a]	2917±833[a]	694±694[a]	5972±4592[a]
Oscillatoria	2222±1470[a]	2778±1137[a]	5278±2373[a]	4306±972[a]
Cyanophyceae	25833±7561[a]	17083±4416[a]	18611±972[a]	22639±5540[a]
Euglena	7222±1944[a]	6250±2097[a]	8472±1211[a]	7639±2046[a]
Phacus	5000±1684[a]	3472±1740[a]	4583±417[a]	4444±1111[a]
Trachalomonas	5417±481[ab]	9306±2572[a]	2917±1102[b]	3056±1806[b]
Euglenophyceae	17639±694[a]	19028±5879[a]	15972±1470[a]	15139±3745[a]
Batrachosperium	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Lemanea	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Rhodophyceae	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Oedogonium	10278±6144[a]	3056±3056[a]	18889±5725[a]	9306±5413[a]
Nodularia	278±278[a]	556±556[a]	0±0[a]	278±278[a]
Tribonema	0±0[a]	0±0[a]	1667±1667[a]	4722±2767[a]
Stigeoclonium	8750±4732[a]	2222±2222[a]	1667±1667[a]	3056±3056[a]
Other	19306±9820[a]	5833±5023[a]	22222±7097[a]	17361±5592[a]
Total Phytoplankton	**161944±14446[a]**	**93333±33208[a]**	**133750±7504[a]**	**135556±17504[a]**
Difflugia	6389±911[a]	4722±367[a]	10139±2900[a]	6389±911[a]
Sarcodina	6389±911[a]	4722±367[a]	10139±2900[a]	6389±911[a]
Asplanchna	5139±1450[a]	3333±241[ab]	1944±735[b]	3889±733[ab]
Brachionus	8194±1690[a]	3472±2183[a]	3472±845[a]	5278±845[a]
Filinia	0±0[a]	0±0[a]	0±0[a]	0±0[a]

Group	Treatment			
	Whole Bamboo	Bottle	Split Bamboo	Banana Stem
Keratella	1528±845^{a}	833±833^{a}	694±367^{a}	1250±722^{a}
Lecane	694±694^{ab}	1528±139^{a}	0±0^{b}	0±0^{b}
Polyarthra	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Trichocerca	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Rotifera	15556±2373^{a}	9167±3182^{ab}	6111±1602^{b}	10417±636^{ab}
Cyclops	1806±605^{a}	972±139^{a}	694±367^{a}	417±417^{a}
Dioptomus	0±0^{a}	0±0^{a}	0±0^{a}	278±278^{a}
Daphnia	278±278^{ab}	1250±636^{a}	0±0^{b}	0±0^{b}
Diaphanosoma	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Moina	0±0^{a}	0±0^{a}	278±278^{a}	0±0^{a}
Nauplius	2639±1002^{a}	1528±367^{a}	694±694^{a}	972±501^{a}
Crustacea	4722±1410^{a}	3750±636^{a}	1667±833^{a}	1667±833^{a}
Total Zooplankton	**26667±3368^{a}**	**17639±4183^{a}**	**17917±4507^{a}**	**18472±1602^{a}**

Table 7. Abundance (no./cm^2) of periphyton in each substrate treatment in Nawalparasi

Group	**Treatment**			
	Whole Bamboo	Bottle	Split Bamboo	Banana Stem
Coscinodiscus	3056±1547^{a}	1806±1410^{a}	3333±1735^{a}	2500±241^{a}
Cyclotella	5833±636^{a}	4722±1959^{a}	6250±867^{a}	8472±2661^{a}
Diatoma	7778±3456^{a}	6389±2504^{a}	6389±2074	7361±2074^{a}
Fragillaria	0±0^{b}	278±278^{ab}	694±367^{a}	0±0^{b}
Gomphonema	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Melosira	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Navicula	6806±3852^{a}	5694±2410^{a}	10556±4938^{a}	6111±2434^{a}
Nitzschia	2778±2778^{a}	4167±2295^{a}	2500±1339^{a}	2778±2778^{a}
Surirella	0±0^{a}	0±0^{a}	0±0^{a}	417±417^{a}
Synedra	3611±1602^{a}	3889±1325^{a}	4538±1502^{a}	3333±1667^{a}
Bacillariophyceae	29861±6974^{a}	26944±5198^{a}	34306±12523^{a}	30972±7500^{a}
Actinastrum	2500±2500^{a}	0±0^{a}	1111±1111^{a}	278±278^{a}
Ankistrodesmus	6667±1049^{a}	6944±1637^{a}	8889±1234^{a}	8889±773^{a}
Chlamydomonas	694±694^{a}	2222±2222^{a}	2361±2361^{a}	278±278^{a}
Characium	556±556^{a}	278±278^{a}	0±0^{a}	556±556^{a}
Chlorella	9583±4829^{a}	11111±3546^{a}	5972±2989^{a}	10556±3729^{a}
Chodatella	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Closterium	0±0^{a}	0±0^{a}	972±605^{a}	0±0^{a}
Coelastrum	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Cosmarium	1528±972^{a}	2361±2361^{a}	1806±1085^{a}	556±556^{a}
Crucigenia	3750±1667^{a}	3889±2286^{a}	5278±735^{a}	5694±3046^{a}
Gonatozygon	0±0^{a}	2500±2500^{a}	2639±1869^{a}	1528±1528^{a}
Mougeotia	1250±1250^{a}	3056±1547^{a}	1806±1085^{a}	833±833^{a}
Oocystis	0±0^{a}	417±417^{a}	0±0^{a}	0±0^{a}

Group	Treatment Whole Bamboo	Bottle	Split Bamboo	Banana Stem
Pediastrum	4861±2572[a]	6667±1684[a]	6528±2504[a]	10139±5576[a]
Scenedesmus	1667±962[a]	1667±1667[a]	2222±1137[a]	3750±833[a]
Selenastrum	694±694[a]	694±694[a]	417±417[a]	0±0[a]
Sphaerocystis	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Staurastrum	694±694[a]	278±278[a]	556±556[a]	833±481[a]
Tetreedron	1250±1250[a]	0±0[a]	833±833[a]	3056±3056[a]
Tetraspora	0±0[a]	694±694[a]	0±0[a]	1389±1389[a]
Treubaria	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Ulothrix	0±0[a]	1111±1111[a]	972±972[a]	0±0[a]
Volvox	0±0[a]	1111±1111[a]	0±0[a]	0±0[a]
Chlorophyceae	35694±9293[a]	45000±7051[a]	42361±5684[a]	48333±13581[a]
Anabaena	5000±2927[a]	5000±3960[a]	6111±4654[a]	3056±2457[a]
Aphanocapsa	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Chroococcus	2917±2917[a]	4861±2457[a]	3611±2819[a]	6111±6111[a]
Gloeocapsa	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Gomphosphaeria	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Merismopedia	5694±2183[a]	2361±1450[b]	3194±1707[ab]	3750±2774[ab]
Microcystis	2500±1735[a]	556±556[a]	2222±605[a]	2361±1187[a]
Oscillatoria	3333±1463[a]	6111±1325[a]	4444±1806[a]	4444±1602[a]
Cyanophyceae	19444±9343[a]	18889±4728[a]	19583±8819[a]	19722±11056[a]
Euglena	5417±636[a]	7778±1325[a]	8333±1869[a]	8056±1869[a]
Phacus	1111±605[a]	1111±735[a]	972±605[a]	1667±636[a]
Trachalomonas	5556±3522[a]	5139±2102[a]	4861±1368[a]	7500±962[a]
Euglenophyceae	12083±2546[a]	14028±1187[a]	14167±1502[a]	17222±3266[a]
Batrachosperium	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Lemanea	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Rhodophyceae	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Oedogonium	10139±6590[a]	20556±1038[a]	25972±17783[a]	10417±5320[a]
Pithophora	0±0[a]	0±0[a]	2083±2083[a]	0±0[a]
Uronema	0±0[a]	0±0[a]	2222±2222[a]	972±972[a]
Other	10139±6590[a]	20556±10348[a]	30278±15696[a]	11389±5700[a]
Total Phytoplankton	**107222±31753[a]**	125417±19731[a]	140694±35210[a]	127639±41629[a]
Difflugia	2639±845[a]	3611±1822[a]	4722±1707[a]	2361±1773[a]
Sarcodina	2639±845[a]	3611±1822[a]	4722±1707[a]	2361±1773[a]
Asplanchna	4167±2295[a]	3194±1211[a]	3056±911[a]	2639±1325[a]
Brachionus	3611±2312[ab]	2917±1102[b]	6111±1773[a]	4722±2650[ab]
Filinia	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Keratella	972±972[a]	278±278[a]	417±417[a]	556±556[a]
Lecane	556±556[a]	278±278[a]	556±556[a]	694±694[a]
Polyarthra	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Trichocerca	0±0[a]	0±0[a]	0±0[a]	0±0[a]

Group	Treatment Whole Bamboo	Bottle	Split Bamboo	Banana Stem
Rotifera	9306±972[a]	6667±636[a]	10139±1002[a]	8611±1470[a]
Cyclops	972±501[a]	833±481[a]	556±556[a]	556±556[a]
Dioptomus	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Daphnia	139±139[a]	0±0[a]	278±278[a]	0±0[a]
Diaphanosoma	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Moina	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Nauplius	833±481[a]	694±367[a]	556±139[a]	556±278[a]
Crustacea	1944±972[a]	1528±845[a]	972±972[a]	1111±735[a]
Total Zooplankton	**13889±911[a]**	**11806±2650[a]**	**15833±1879[a]**	**12083±3014[a]**

Table 8. Periphyton biomass in four substrate treatments

	Whole Bamboo	Bottle	Split Bamboo	Banana Stem
		Chitwan		
Dry matter (g/cm^2)	0.0200±0.0019[a]	0.0260±0.0070[a]	0.0244±0.0024[a]	0.0188±0.0073[a]
Ash content (g/cm^2)	0.0130±0.0011[a]	0.0193±0.0053[a]	0.0168±0.0022[a]	0.0124±0.0055[a]
Ash free dry matter (g/cm^2)	0.0070±0.0010[a]	0.0068±0.0018[a]	0.0076±0.0011[a]	0.0063±0.0019[a]
		Nawalparasi		
Dry matter (g/cm^2)	0.0271±0.0099a	0.0409±0.0056a	0.0292±0.0068a	0.0432±0.0123a
Ash content (g/cm^2)	0.0157±0.0071a	0.0313±0.0044a	0.0205±0.0061a	0.0337±0.0107a
Ash free dry matter (g/cm^2)	0.0114±0.0031a	0.0095±0.0012a	0.0087±0.0010a	0.0095±0.0025a

Fish production was lower in Chitwan because a flood hit the district in August 2017, when flood water entered the ponds and swept away fish. Dikes also were damaged and fish escaped. Villagers caught pond fish from rice fields and roads later (see picture in Figure 1). On final harvest, 0 to 315 fish were obtained from the ponds in Chitwan. There were no fish in the pond of Ranjita Mahato, while 2, 5, and 9 fish were harvested from ponds of Saraswati Chaudhary, Bijaya Chaudhary, and Gunja Chaudhary, respectively, even though the number of stocked fish ranged from 71 to 824. Highest combined net fish yield was found in control ponds (2.83 $t{\cdot}ha^{-1}{\cdot}yr^{-1}$) and lowest in banana midrib ponds (0.96 $t{\cdot}ha^{-1}{\cdot}yr^{-1}$), corresponding to carp survival of 40% and 26%, respectively (Table 9). Final carp biomass ranged from 6.2 kg/100 m^2 in banana midrib ponds to 17.4 kg/100 m^2 in control ponds after 230 days. Final SIS weight ranged from 0.4 kg/100 m^2 in banana midrib ponds and plastic bottle ponds to 1.0 kg/100 m^2 in control ponds. Stocked weight of SIS was not accounted because farmers did not weigh SIS while stocking, they just let them enter along canal water through water inlet. Feed conversion ratio varied from 1.7 to 39.4.

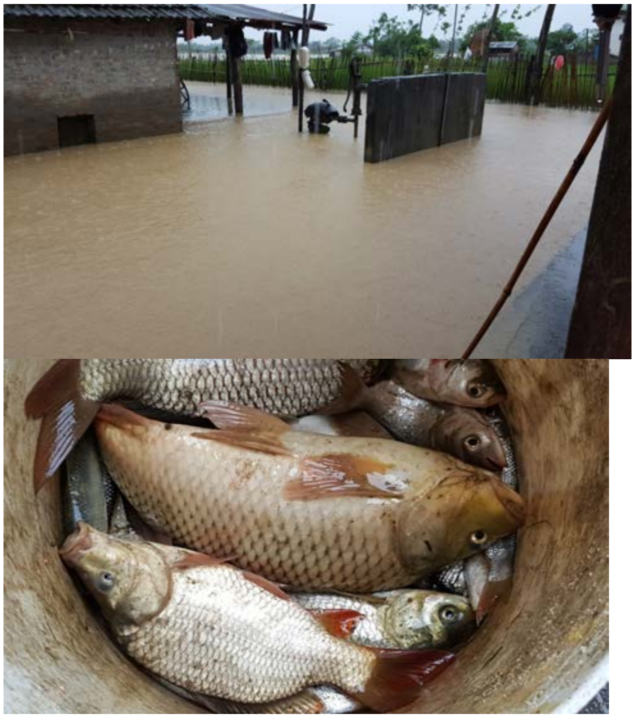

Figure 1. Inundated house of Maya Chaudhary and fish caught in the road in front of her house.

Table 9. Yield of carp and SIS (kg/100 m^2) in each treatment after 230 days in Chitwan and 210 days in Nawalparasi.

	Control	Split Bamboo	Whole Bamboo	Banana midrib	Bottle
Chitwan					
Initial Mean Carp Weight (g/fish)	4±0.2	3.9±0.0	3.9±0.0	3.9±0.0	3.9±0.0
Initial Carp Weight (kg/100 m^2)	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0
Initial Total Weight (kg/100 m^2)	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0
Final mean Carp Weight (g/fish)	258±89	367±79	228±36	180±19	138±70
Final Total Carp Weight (kg/100 m^2)	17.4±8.9	10.1±9.5	12.3±0.5	6.2±3.1	10.7±5.6
Final Total SIS Weight (kg/100 m^2)	1.0±0.2	0.8±0.3	0.6±0.3	0.4±0.1	0.4±0.2
Final Combined Total Weight (kg/100 m^2)	18.4±0.8	11.0±9.4	12.9±0.5	6.6±3.0	11.0±5.7
Combined Total weight gain (kg/100 m^2)	17.8±8.9	10.4±9.4	12.3±0.5	6.0±3.0	10.5±5.7
Survival (%)	40±8	13±16	38±7	26±12	34±17
Combined GFY (t·ha^{-1}·yr^{-1})	2.93±1.40	1.74±1.49	2.05±0.08	1.05±0.48	1.75±0.91
Combined NFY (t·ha^{-1}·yr^{-1})	2.83±1.41	1.65±1.49	1.96±0.08	0.96±0.48	1.66±0.91
Feed Conversion Ratio	3.8±1.7	39.4±79.9	1.7±0.3	8.1±3.5	15.8±17.4
Nawalparasi					
Initial Mean Carp Weight (g/fish)	3.9±0.0^a	3.9±0.0^a	3.9±0.0^a	3.9±0.0^a	3.9±0.0^a
Initial Carp Weight (kg/100 m^2)	0.6±0.0^a	0.6±0.0^a	0.6±0.0^a	0.6±0.0^a	0.6±0.0^a
Initial Total Weight (kg/100 m^2)	0.6±0.0^a	0.6±0.0^a	0.6±0.0^a	0.6±0.0^a	0.6±0.0^a
Final Mean Carp Weight (g/fish)	218±36^a	229±17^a	228±22^a	253±10^a	260±16^a

	Control	Split Bamboo	Whole Bamboo	Banana midrib	Bottle
			Chitwan		
Final Total Carp Weight (kg/100 m^2)	20.9±2.8[a]	22.3±1.5[a]	23.3±4.3 [a]	24.6±0.7[a]	24.9±2.1[a]
Final Total SIS Weight (kg/100 m^2)	0.4±0.0[a]	0.4±0.1[a]	0.5±0.1[a]	0.6±0.1[a]	0.4±0.1[a]
Final Combined Total Weight (kg/100 m^2)	21.3±2.8[a]	22.6±1.6[a]	23.9 4.3[a]	25.2±0.6[a]	25.4±2.2[a]
Combined Total weight gain (kg/100 m^2)	20.7±2.8[a]	22.0±1.6[a]	23.2±4.3 [a]	24.5±0.6[a]	24.7±2.2[a]
Survival (%)	67±3[a]	67±6[a]	69±6[a]	69±4[a]	69±7[a]
Combined GFY (t/ha/yr)	3.70±0.4[a]	3.93±0.28[a]	4.15±0.74[a]	4.38±0.10[a]	4.41±0.37[a]
Combined NFY ($t{\cdot}ha^{-1}{\cdot}yr^{-1}$)	3.59±0.49[a]	3.82±0.28[a]	4.03±0.74[a]	4.27±0.10[a]	4.30±0.37[a]
Feed Conversion Ratio	2.0±0.0[b]	1.5±0.1[a]	1.7±0.2[ab]	1.8±0.1[ab]	1.9±0.2[ab]

Overall fish production was highest in the treatments with plastic bottles or banana midribs as substrate (Table 9). Unfortunately, the flooding in Chitwan resulted in such a disruption to fish production that we could not determine treatment effects at that location. For Nawalparasi, final total weight of carp and combined NFY was higher in substrate ponds than control ponds, indicating that rohu and common carp utilized periphyton for food. Both final total weight of carp and combined NFY was 19% higher in ponds with plastic bottles than control ponds. Final total weight of SIS in banana midrib ponds was 50% higher than control, strip bamboo, and plastic bottle treatments. Feed conversion ratio was lower than 1.9 in all substrate ponds. FCR was significantly lower ($P<0.05$) in split bamboo ponds than control ponds but it was similar to values in other substrate ponds.

Gross margin and gross return were highest in the plastic bottle and banana leaf treatments, but due to variability from the flooding, none of these results were statistically significant. Once again, we could not determine these values accurately for Chitwan. Feed cost was less than NRs 866 in substrate treatments in Chitwan because ration was reduced after assessing fish biomass in each pond after flood (Table 10). Related to feed cost, total variable cost was also low in all substrate treatments. Feed cost ranged from NRs 1066 in split bamboo ponds to NRs1489 per 100 m^2 in 210 days in banana midrib ponds while total variable cost ranged from NRs 2124 in split bamboo ponds to NRs 2509 per 100 m^2 in 210 days in banana midrib ponds in Nawalparasi. Both gross return and gross margin were higher in substrate ponds than control ponds. Gross return was 19% higher in plastic bottle treatments than controls while gross margin was 30% higher in plastic bottle treatments ponds than controls.

Table 10. Gross margin (Rs/100 m^2 pond) analysis for each treatment after 230 days in Chitwan and 210 days in Nawalparasi.

	Control	Split bamboo	Whole bamboo	Banana leaf	Bottle
			Chitwan		
Cost					
Carp fingerlings	494±5	487±1	488±0	485±1	487±0
Lime	72±0	72±0	72±0	72±0	72±0
Urea	107±2	112±2	112±1	110±1	112±0
DAP	212±8	217±4	218±1	222±1	220±20
Feed	1164±59	855±70	660±115	866±89	842±124
Total Variable Cost	2049±56	1745±68	1549±115	1756±90	1734±106
Return					
Carp	4697±2408	2738±2559	3315±127	1672±847	2879±1506
SIS	208±38	164±52	125±68	81±19	75±32

	Control	Split bamboo	Whole bamboo	Banana leaf	Bottle
Gross Return	4905±2391	2902±2538	3440±131	1753±829	2954±1537
Gross Margin	2856±2445	1157±2479	1890±103	-2±822	1220±1561
			Nawalparasi		
Cost					
Carp fingerlings	488±0	487±1	487±0	488±0[a]	488±0
Lime	72±0	72±0	72±0	72±0	72±0
Urea	164±1	166±0	166±0	165±1	166±0
DAP	329±1	333±2	330±1	331±1	245±89
Feed	1368±192 [a]	1066±75[a]	1306±288[a]	1452±117[a]	1489±25[a]
Total Variable Cost	2422±193[a]	2124±74[a]	2362±287[a]	2509±115[a]	2460±111[a]
Return					
carp	6258±846[a]	6678±461[a]	6993±1281[a]	7386±206[a]	7485±636[a]
SIS	83±0[a]	75±17[a]	109±26[a]	115±27[a]	87±13[a]
Gross Return	6342±846[a]	6753±473[a]	7102±1283[a]	7501±180[a]	7572±643[a]
Gross Margin	3920±655[a]	4630±440[a]	4741±1062[a]	4992±164[a]	5111±749[a]

DISCUSSION

The four treatments for substrate resulted in considerably higher fish production and gross margin than the control without substrate. Fish production was calculated by summing weight of fish consumed by farmers and weight of fish netted during final harvest. Among substrates used, plastic bottles gave higher fish yield than natural substrates which differed from results obtained in previous work, where natural substrates such as bamboo produced higher fish yield (Van Dam et al., 2002). Most likely differences in the surface area of each substrate type, the exposure to sunlight, and the attraction of algae to the substrate surface made each substrate type a unique environment for production of periphyton and the resulting difference in fish production. Better FCR in substrate ponds than control ponds in Nawalparasi showed that periphyton reduced feed input and feed cost. FCR was much better in the present trial than in the previous field trial as reported by Jha et al. (2018) in the same place.

Fish production was affected by flood in both districts, but the effect of flooding was more serious in Majhui, Chitwan than in Seri and Nandapur, Nawalparasi. There is a stream very close to Majhui that exceeded its banks due to continuous intense rainfall, and water inundated the village. Water entered the ponds and fish escaped. Moreover, the height of dikes for ponds in Majhui was also lower than Nawalparasi, so fish escaped easily. Physical damage also occurred to ponds in Majhui. It took almost three days for flood water to recede in the village. Ponds were netted to assess status of fish nearly one month later. High water level in the pond interfered with netting and fish assessment was not effective. Hence, our data on production and economics does not give real picture of farmers' ponds in Chitwan. The situation was better in Nawalparasi because survival and production of carp was satisfactory and a treatment effect could be seen. But the variation in ponds even there probably resulted in the lack of statistical significance for any of these results. Around 67 to 69% of stocked carp were recovered from ponds there, which is comparable with data from Jha et al. (2018). FCR was very high except for ponds with substrates of whole bamboo in Chitwan, again due to flooding. We could not assess number and weight of fish in ponds to adjust ration after the flood. Due to delay in our fish assessment and farmers putting effort in rehabilitation rather than fish and pond sampling, farmers fed randomly. This hiked feed quantity and FCR in Chitwan.

Gross return and gross margin was very low in all treatments in Chitwan while farmers received good return and margin in Nawalparasi. In Chitwan, 6 farmers (Control-1, split bamboo-2, banana midrib-2, plastic bottle-1) lost money from ponds due to low production caused by the flood. Feed cost and total variable cost was higher in Nawalparasi than Chitwan because survival of carp was higher in Nawalparasi ponds and feed adjustment was done according to fish weight.

Among substrates used, farmers complained about using banana midrib because they had to replace it 3-4 times during the trial period. Banana midrib decayed in ponds within 2 months which created trouble for farmers. Although banana is easily available from the farm and has multiple uses, replacement effort is important and care should be given on use of it because its decay may cause oxygen depletion in the pond. Comparatively, whole bamboo, split bamboo and plastic bottles are more durable. Based on fish production, profit, and availability, plastic bottles appear to be a reasonable alternative to split bamboo mats for periphyton substrate.

Workshop for Non-Adopting Farmers

A workshop for non-adopting farmers was conducted at Hotel Gangotri, Narayangarh, Chitwan, on 8 January 2018. The objective of the workshop was to disseminate the results of the field trial. Altogether 42 participants including 31 from Chitwan and 11 from Nawalparasi participated in the workshop. Among them, 41 were farmers and 1 was NGO Chair (Rural Integrated Development Society). By gender, 62% of participants were female while 38% were male, while 54% were from Chitwan and 7% from Nawalparasi.

Table 11. Participants of the workshop for non-adopting farmers

Participants	Chitwan			Nawalparasi		
	Male	Female	Total	Male	Female	Total
Farmer	7	23	30	8	3	11
NGO	1		1			
Total	8	23	31	8	3	11

During the workshop, periphyton technology and results of the on-farm trial were presented. The presentation was followed by group discussion on the effectiveness of different substrates and adoption of the technology. Farmers were divided into three discussion groups. After the discussion, each female lead reported on their discussion. Farmers' perceptions on periphyton-based technology and its effectiveness are summarized in Table 12.

Table 12. Outputs of workshop for non-adopting farmers

Particular	Group I	Group II	Group III
Periphyton technology	useful	useful to small scale farmers because this technology helps in reducing pond inputs and increasing production and income	Good for small scale farmers as it helps in reducing feed cost in ponds.
Appropriate substrate	Plastic bottles because they are easy to use and durable for many culture cycles Banana leaves were consumed by grass carp but the midrib could be left in the pond as substrate	Plastic bottles tied in a ring of hollow plastic pipe would be better since this is easier to build and helps in pollution control by using waste material	Whole bamboo with no leaves would be better as it is available, easy to use, and better for periphyton growth. Using whole bamboo with its branches also helps in reducing predation and theft.
Effectiveness/ adoption	Effective and adoptable	Adoptable but needs technical help from AFU	Adoptable but needs technical help
Constraints	No	Exposed parts of substrates may become place for birds to prey on small fish.	Substrates cause problems during partial harvesting of SIS and sampling fish. They may also cause some wounds in fish.
Solution		Gill net may be placed on the ring of pipe to stop birds from using substrate as resting place	

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WATER, WATER QUALITY, AND POND BOTTOM SOIL MANAGEMENT IN UGANDAN AQUACULTURE

Production System Design and Best Management Alternatives/Study/16BMA05AU

Shamim Naigaga[1], Claude E. Boyd[1], Joseph J. Molnar[1], and John Walakira[2]

[1]Auburn University, USA
[2]National Fisheries Resources Research Institute (NaFIRRI), Uganda

ABSTRACT

Fisheries production from lakes and other natural waters in Uganda are declining and expansion of the aquaculture sector is needed to increase aquatic protein for human consumption. The present study was conducted to improve understanding of limitations imposed on aquaculture by the environmental factors of climate, soils, and water quality in Uganda. Although not optimum, the temperature regime in Uganda is conducive to year-around aquaculture in most areas. Rainfall is adequate to maintain water levels in ponds throughout the year in the Western, Eastern, and Central regions. In the Northern region, it would be necessary to store water in farm reservoirs to use for maintaining water levels during the driest months. There is a tendency towards drought in Uganda, and severe droughts could cause water shortages for aquaculture. Water quality was generally suitable in all four regions for fish production. The main limitation would be the need to lime ponds in some areas in all regions. Unfortunately, the liming materials available in the country are of poor quality, and the agricultural limestone currently used by fish farmers is particularly low in quality. There is an urgent need to find better sources of liming materials and begin an effort to promote liming in Ugandan aquaculture. The main limitations of soils for pond sites were coarse soil texture, steep terrain in some areas, and a widespread problem of low acidity. Of course, as in any country, each prospective pond site must be examined for its suitability. There also is cage culture in several lakes in Uganda; Lake Victoria and Lake Albert appear to be the best lakes in which to consider expansion of cage culture operations. In summary, there does not appear to be insurmountable environmental restrains to expanding aquaculture production in Uganda. The major issues relate to selecting good sites for ponds and to finding a source of good quality liming material.

INTRODUCTION

Capture fisheries in Uganda have declined, and there is interest and apparent potential for increasing the production of fisheries product through expansion of the existing aquaculture sector. The present study builds on the previous investigation of the use of water quality test kits in Uganda (Naigaga et al., 2015) to expand knowledge of water quality characteristics of source water for pond aquaculture and suitability of water in the major lakes for cage culture.

In addition, the present study assesses pond soil characteristics and climatic conditions as they affect pond hydrology and fish production, and examines the quality of liming materials available for use in Ugandan ponds. This information is used to provide more details about the potential for aquaculture and methods of pond water quality management that may be necessary.

OBJECTIVES

The specific objective for the study are as follows:

1. Measure water quality in reservoirs, lakes, and ponds, ponds soil characteristics in relation to climatic conditions, pond hydrology, and quality of liming materials.
2. Develop generalized water budgets for rain-fed ponds to ascertain likely variation in water levels.

3. Estimate water inputs necessary for ponds filled from external sources.
4. Evaluate the need for liming and the quality of local liming materials.
5. Assess water quality amendments required in aquaculture ponds.

METHODS AND MATERIALS

Auburn University doctoral student Shamim Naigaga traveled to major aquaculture areas in Uganda, specifically ponds, reservoirs, and lakes to collect water samples. In Uganda, the Aquaculture Research and Development Center-Kajjansi supported the data collection. The study sampled 120 pond water sources and the five major lakes in Uganda (Victoria, Albert, Kyoga, Edward, and George).

The pH, temperature, and dissolved oxygen concentration were measured at time of collection. There was no time allocation for sampling pond water sources but, for the lakes, sampling was between 8 am to 1 pm before diel changes.

Samples of 1L each in plastic bottles were shipped to Auburn University for measurement of specific conductance, alkalinity, hardness, major ions, chemical oxygen demand, and trace elements by standard protocol (Eaton et al., 2005).

Soil samples were collected from five locations in each pond, samples from each pond were combined into a composite sample, dried at 60°C in an oven, and shipped to Auburn University. The samples were analyzed for pH, organic carbon, cation exchange capacity, phosphorus, nitrogen, major ions, trace metals, free carbonate, and particle size.

Weather records were obtained from four locations: Gulu, Mbarara, Jinja and Soroti. The records contain monthly average temperatures, monthly maximum and minimum temperatures, rainfall, and if possible, pan evaporation. The water loss rate was measured from five to ten representative ponds over a period of 2-4 days without rainfall with aid of a stilling well and hook gauge (Yoo and Boyd, 1994).

Five samples of liming materials (200 g each) used in Uganda were collected and analyzed for neutralizing value and particle size at Auburn University.

Information was collected on typical production levels in ponds, amounts of fertilizers, liming materials, feeds and other inputs. The use of aeration in ponds were noted. If aerators are used, information on type, size, and typical operating schedule of aerators were obtained. The data were used to assess the suitability of water and soil quality for aquaculture in Uganda. This assessment includes the following:

- Generalized water budgets for rain-fed ponds were estimated to ascertain likely variation in water levels. Estimates of water inputs necessary for ponds filled from external sources also were made.
- The need for liming were evaluated, and the quality of local liming materials were determined.
- An assessment of other water quality amendments that might be required in ponds were made.
- Climatic variation over the country were assessed for possible effects on aquaculture.
- The assessment results were used to prepare recommendations for water, water quality, and pond bottom soil management in Ugandan aquaculture.

RESULTS

Climate

Mean temperature and mean annual rainfall was higher for the Northern and Eastern regions than the Central and Western regions (Table 1), respectively. There were significant differences between the mean temperature ($F= 836.06$, $p < 0.0001$, $df = 3$, at $p = 0.05$) and mean annual rainfall ($F = 41.66$, $p < 0.0001$, $df = 3$, at $p = 0.05$) among the regions (Table 2).

The post-hoc multiple comparison test by Tukey's Studentized range (HSD) showed mean temperatures were different among regions and mean annual rainfall was also different among regions apart from that of Eastern and Central regions which were similar (Table 2).

Linkages between temperature and optimum temperature range for catfish and tilapia showed that regions other than the Eastern region had mean temperature below the optimum range of 26°C to 32°C (Azaza, et al., 2010) over the years of record (Figure 2).

Seasonal Mann-Kendall output showed, apart from the Eastern region, a positive increasing trend in temperature among the regions (Table 3 and Figure 2). The strength of the slope was weak for the regions that have the trend, as the temperature varied between 0.002° C/yr for the Eastern region to 0.058°C/yr for the Northern region (Table 3). Deviations from the long-term mean (Figure 3) showed that there were higher temperature fluctuations in the Western region than in other regions over the years.

The 12-month Standard Precipitation Index (SPI) (Table 4) revealed a rainfall deficit in the Northern and Western regions, but the deficits were not great enough to be considered outside the normal range in SPI according to guidelines for classifying rainfall deficits given by Mckee et al. (1993). Eastern and Central regions were also near normal, although the Central region had the highest SPI (Table 4). Over the years, all regions fell within -1.5 to 1.5 SPI resulting in classification varying between moderately dry to moderately wet (Figure 4).

Mean evaporation was higher in the Northern region, followed by the Central region, and least in the Western region, but there were missing data for the Eastern region (Table 5). Calculated monthly potential evapotranspiration was highest for the Eastern region, followed by the Northern region, then the Central region, and least potential evaporation was for the Western region (Table 5). On a yearly basis, the potential evapotranspiration was lower than the evaporation estimated for ponds in all regions.
The Western region had the lowest water requirement compared to other regions (Figure 5) despite having the lowest mean SPI. The monthly required inflows for levee ponds showed that all regions required water during the December-February period, which is the longest dry season in all but the Western region where the dry season typically is only during July (Table 6). Nevertheless, the Northern region had the highest water requirements of the four regions.

Water quality

Pond mean concentrations of the different water quality parameters showed that most were within optimum ranges for Nile tilapia and African catfish. The exceptions were in the Central region for total alkalinity and in the Eastern region for pH (Table 7). However, the ranges of each water quality parameter measured were wide. The Northern region had the highest water hardness, total alkalinity and associated buffering capacity, and the greatest specific conductivity as compared to other regions.

Comparisons among the different regions by a chi square test (Table 8) showed that all regions were independent of each other as p-value was less than $\alpha = 0.05$, $df = 3$ at 95% confidence interval. The

ANOVA output showed that mean concentrations for all water quality parameters analyzed were statistically significantly other than for the calcium hardness ($p = 0.0828$, $\alpha = 0.005$, $df = 3$) (Table 9). Further comparison with the Tukey's test revealed that the regions had pH statistically significantly different from each other, while for other water quality parameters, the Northern region was statistically different from other regions other than the Eastern region (Table 9).

Pond frequency distributions for water quality variables (Figure 6) showed that most pond water sources had total hardness concentration out of the optimum range for Nile tilapia and African catfish. The Eastern region had most of its water sources within range as compared to other regions, while the Northern region had most water sources with hard water. Pond frequency distribution for specific conductivity (Figure 7) showed that most water sources were within optimum range for the Eastern region when compared to other regions, however, the specific conductivity concentrations in all regions were within the tolerable range as freshwater species which typically can tolerate up to 5,000 µS/cm (Boyd and Tucker, 2014).

Total alkalinity pond frequency distribution (Figure 8) showed that most water sources for all regions were below the optimum range, except for the Eastern region. The Central region had lowest total alkalinity concentrations when compared to other regions. Pond frequency distribution for pH (Figure 9) showed that regions other than the Eastern region had water sources within the optimum pH range. This observation agrees with studies done by Boyd and Tucker (2014), in which ponds with total alkalinity below 50 mg/L usually had pH between 6 to 8 when sampled in the morning.
Pond trace metal analysis revealed that concentrations of most trace metals were within optimal limits for fish culture. The exceptions were aluminum and iron (Table 10), which sometimes were greater in concentration than the normal concentrations listed for freshwater pond in Boyd and Tucker (1998) and (Boyd, 2015). This also agreed with studies which showed elevated concentrations of iron and aluminum in ground waters of Uganda (UNESCO, 2006).

Frequency analysis of iron concentrations showed that regions other than the Western region had at least 50% of most water sources in the optimal range. This was contrary to the study done on drinking water in Western region by Ngabirano et al. (2017), which showed that most of the water sources analyzed had iron concentrations within optimal range. For the aluminum concentration, the Northern and Western regions at least 50% of the water sources were in the optimum range as compared to Central and Eastern regions (Table 11).

Lake results showed that Lakes George and Kyoga were poor sites for fish cage culture, Lake Edward had its siting as fair, with Lakes Victoria and Albert having suitable site characteristics based on the following parameters: water depth, secchi depth, dissolved oxygen profile, long axis to the bay, currents, connectivity to open water, and distance to pollution sources (Table 12). Further analysis of other water quality parameters of concern in aquaculture (Table 13) showed that these lakes were in optimal range for cage culture. Nonetheless, the low calcium concentration indicated by calcium hardness <20 mg/L in Lake Victoria can be compensated by calcium in the artificial feed given to the fish.

Soil characteristics
The mean soil pH values for the different regions were below the optimum range for fish production (Table 14), with the Central region having the lowest average soil pH. The percentage soil total nitrogen was optimal for all regions, while the percentages of total soil carbon and soil organic matter were within optimum range for all regions other than the Western region. The saturated hydraulic conductivity was higher for the Western region as compared to other regions (Table14).

A chi square test (Table 15) showed that all regions were independent of each other.

The ANOVA output revealed that mean concentrations for all soil quality parameters were statistically significant among the four regions (Table 16). On using Tukey's test (Table 16), soil pH of the Central and the Western regions were statistically different from that of Eastern and Northern regions. For other soil quality parameters, the Western region was statistically different from other regions.

Frequency distribution of soil pH showed that most ponds had a soil pH out of the acceptable range, with the Central and Western regions having the highest number of ponds with a pH less than 6.5 (Figure 10). However, none of the soil samples from any region had a soil pH above the highest recommended soil pH for fish culture.

In most of the ponds, the percentage total carbon content was within the optimal range and especially in the Central and the Northern regions (Figure 11). However, the Western region had most of the ponds that with a carbon content percentage above the recommended level.

Frequency distribution of the organic matter content in soil samples in the different regions revealed similar trends in frequency distribution. The Northern region had the greatest frequency of soil samples within the optimal range and the Western region having most soil samples with elevated organic matter content (Figure 12).

All trace metal concentrations were within the optimal range, apart from the copper concentrations for which some soil samples were above the maximum safe concentration of 2 mg/kg for copper in sediment reported by Abdul-Wahab and Jupp (2009) (Table 17). However, this reference refers to copper in subtidal, estuarine marine environments and unlikely applies well to aquaculture pond soils. Nevertheless, the Northern and Eastern regions had a number of soil samples with copper concentration higher than the recommended range reported for estuarine sediment.

Liming requirement results inferred from the soil pH and buffer pH showed that the Central region had the highest number of ponds (>80%) that required liming and the Northern region the least number of ponds that required liming (60%) (Figure 13). However, a different trend was shown with the lime requirement of the ponds that required liming with onds in the Western region having a higher average liming requirement of 2970 ± 414.42 kg/ha or 743 ± 103.61 kg/pond area (500 m^2) (Table 18). The Northern region still had the least lime requirement as many soils there contain limestone.

The neutralizing value and fineness rating are used to assess the quality of agricultural limestone, but only the neutralizing value is used for burnt and hydrated lime as lime is more soluble than agricultural limestone (Boyd and Tucker 2014). Data in Table 19 reveal that the ENV or effective neutralizing value [(NV % × FV %) ÷ 100] of the four available liming products obtained in Uganda ranged from 8.9 to 51.8%.

According to Table 19, the agricultural limestone products would be very inefficient for use in ponds because a huge quantity would be necessary. The best choice seems to be the Neelkanth Ltd hydrated lime, but it cannot be applied at over 200 kg/ha per application in ponds with fish, because lime can raise pH above the level tolerated by fish (Boyd and Tucker, 2014). As a result, liming would have to be done to the pond bottoms between crops and 2 to 3 weeks allowed for the pH to fall after refilling ponds and before stocking fish.

Liming rates for the best liming material (Neelkanth Ltd hydrated lime class A) were very high for all regions (Table 20). Liming rates followed a similar trend as lime requirement with the Western region having a higher liming rate, followed by the Central region, then the Northern region, and least for the Eastern region.

Observed seepage rates results showed that the Western region had the least seepage rate (0.93 ± 0.17 cm/hr) and the Central the highest seepage rate (3.37 ± 2.03 cm/hr) (Table 21). These results were contradictory to the calculated seepage rates from the saturated hydraulic conductivity, which resulted in the Northern region with least seepage rate, followed by the Eastern region, then the Central region, and finally the Western region. The Western region had higher seepage rates which could be due to the organic soils (Egna and Boyd, 1997). The calculated seepage rates were higher than the observed seepage rates.

DISCUSSION

Climate

The mean annual temperatures and mean annual rainfall totals observed in the different regions agreed with a study done by Phillips and McIntyre (2000). Higher mean rainfall for the eastern region can be attributed to Lake Victoria and Lake Kyoga's influence on the moisture availability in the local atmosphere (Sun et al., 2015). Similarities in the mean annual rainfall of the Central and Eastern regions were in line with data reported by Nicholson (2017) which allowed the Central and Eastern regions to be classified as being in the equatorial rainfall region.

Although the temperatures were out of the optimum range for the culture of African catfish and Nile tilapia, they were within the range for optimum feeding by these species which has a lower limit of 20°C (Azaza et al., 2010; Isyagi et al., 2009). The lower temperature in the Western region possibly could allow culture cold-water fish species, such as trout, in some areas.

The positive trend of temperatures in the different regions by the seasonal Mann-Kendall implies increasing temperatures in those regions which favor aquaculture production in the future. Deviations from the long term mean in the Western region was, however, within 6°C for all regions. This was noted to be suitable for young fish, but not larger fish (Azaza et al., 2010). The negative SPI values in the Northern and Western regions indicate the need for strategies for storing water in reservoirs during the rainy seasons for use in the dry season.

The low water requirement in the Western region, which has a low SPI, likely is the result of lower potential evapotranspiration, and seepage rate in the Western region compared to other regions. The high-water requirement observed in the Northern region has a unimodal rainfall pattern compared to other regions, which have a bimodal rainfall; hence, more precipitation occurs and increases the water table throughout the year. The Northern region is also far away from the influence of the moisture transport from Lake Victoria resulting in drier climate. Therefore, a year-round production cycle is possible in the Western region that would allow for stocking ponds at different times, however, it is best when pond preparation is in June-July when pond bottoms dry out and fertilizer application can be made in the dry season without nutrient leaching problems. The Eastern and Central regions had a similar possible production cycle where pond preparation or fish harvest could be in June-July or December-February. This is advantageous as pond refilling would be easier during the rainy season that follows, and it also presents a competitive market and high profit margin because many poultry products are less available in the local markets during dry season (Maurice et al., 2010).

In the Northern region, a single production cycle is possible with pond preparation in March and fish harvest in December unless water harvesting is done to store water for use during the dry months. Fish harvest in December would be advantageous to the Northern region as fish is a delicacy sought at this time of the year (Jagger and Pender, 2001), and fish harvest during the holiday and vacation season increase profits for the farmers.

Considering all factors, the Western region had the most favorable climate for aquaculture production, followed by the Eastern, then the Central, and finally the Northern region. However, the topological issues associated with the Western region noted by Ssegane et al. (2012), results in the Eastern region being the most favorable for aquacultural production. Nevertheless, in the context of water and soil quality, all regions are suitable for aquaculture production, provided water harvesting strategies are adopted for storing water during the dry periods.

Water quality
The high concentrations of specific conductivity, total alkalinity, and total hardness in the Northern region agree with studies done by Boyd & Tucker (2014) in which moderate to high concentrations of total alkalinity and total hardness were positively correlated with increasing specific conductivity. The Northern region is drier than other regions; hence, more evaporation occurs leading to concentration of ions in water (higher specific conductivity). Similarities between the Northern region and the Eastern region for the mean concentrations of the water quality parameters analyzed could be attributed to similar geological conditions, land uses, and pond management practices.

Higher pH values in the water sources in the Eastern region despite their high total alkalinity could be attributed to excessive photosynthesis by water plants in the ponds as reported to occur in ponds in other parts of the world (Boyd and Tucker, 2014). Most of the water sources in the Eastern region were measured in ponds, and the time of sampling which was done in the early afternoon was at the time of day when pond pH is greatest.

The elevated concentrations of iron and aluminum in the pond water sources could be the result of corrosion of the borehole casings, seepage of the sewage waste and natural weathering of the aquifer matrix which is high in iron and aluminum. Higher occurrence of high aluminum and iron concentrations in the Central and the Eastern regions compared to the Western and the Northern regions could be attributed to the seepage of sewage waste in the Central and Eastern regions, as they have more industries compared to other regions.

Soil characteristics
The soil pH values in all regions were similar to those previously observed in Bogor (Indonesia) by McNabb et al. (1990), who noted that ponds with low pH had low fish production. The high saturated hydraulic conductivity in the Western region is due to the high organic matter in the ponds in the Western region (Hillel, 2008; Oosterbaan and Nijland, 1994). Higher total carbon and organic matter percentages in the Western region possibly can be attributed to the high fertilization rates with manure (Egna and Boyd, 1997).

High copper concentrations above 2 mg/kg in pond soils are fairly common and apparently of no concern (Boyd and Tucker 1998).

All the liming materials analyzed had a very low neutralizing value (NV) and fineness value (FV), which meant higher liming rates (Boyd and Tucker, 2014). Neel-Kanth hydrated lime class A was the only Ugandan liming product with NV and FV similar to those found for pulverized seashells (Boyd and Tucker 2014). Good quality agricultural limestone should have an ENV above 80%, while ENV for lime should be 110%-150% or more (Boyd and Tucker, 2014).

The quality of liming material, and especially of agricultural limestone, in the market in Uganda were extremely substandard. The availability of good quality liming material appears to be a serious limitation to improving pond management in Uganda. Many ponds in the country need to be limed, but the quality of the available products for liming is poor. The cost of agricultural limestone – in spite of its low quality – is high and if quality is considered, the cost of all of the liming materials is outrageous as compared to the costs of these materials in other countries with substantial aquaculture sectors.

The government or private agricultural vendors in Uganda could possibly import liming materials from other countries. It also seems that an effort to improve the quality of the domestic products could be initiated, and possibly there are better liming products which were not located during the present study. Nevertheless, there seems to be good reason to conduct further investigation into this issue.

The seepage rates were all acceptable in aquaculture (Yoo and Boyd, 1994). The seepage rates must be considered approximate because of the crude method used for its determination and the fact that the observed seepage was determined for only three ponds in each region because of time restraints.

It should be mentioned that factors other than the hydraulic conductivity of soil as measured in the laboratory affect seepage rate. During pond construction, soils are compacted to reduce seepage, and organic matter produced in ponds and added to ponds in manure during aquaculture production tends to fill the interstitial spaces among soils to lessen seepage (Yoo and Boyd, 1994). Thus, soil hydraulic conductivity should only be viewed as potential seepage, and soils that have a high potential for seepage should be given special attention for thorough compaction during construction. Of course, with improper construction, a pond constructed on soil with low hydraulic conductivity may seep badly.

CONCLUSIONS

The temperature was sometimes out of the optimum range for production in all regions, but the temperature range would allow fish production. The Western region was considered most favorable among all regions, but it has a steep topography. The Eastern region as the most favorable conditions in Uganda for fish production. However, all areas were noted to be suitable for fish culture, provided water harvesting techniques were employed during the dry period to store water for use in dry weather. Overall, potential climate effects on aquaculture is not that significant in the country if the right production strategies are adopted.

Pond mean concentrations of the different water quality parameters showed that they were within optimum range for African catfish and tilapia culture, but frequency analysis showed otherwise with a high percentage of water sources in all regions being outside of optimal ranges in alkalinity and pH. There is, therefore, a need to analyze water sources from all sites and make applications of agricultural lime to ponds as necessary to increase the pH and alkalinity and favor greater fish production. The Eastern region had better water sources for fish farming as compared to the other regions.

All regions had soil pH below the optimum range for fish culture; hence, liming were required to increase production, especially since most farmers do not apply fish feed. The Northern region generally had the best soil for pond bottoms and the Western region had the most soils with limitations for use as pond soils. All liming materials were of low quality as indicated by low neutralizing value of both agricultural limestone and large particle size distribution in agricultural limestone.

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TABLES AND FIGURES

Table 1: Mean temperature, maximum, minimum temperatures, 1980 to 2016 by region.

	Temperature			Rainfall
Region	**Mean (°C)**	**Minimum (°C)**	**Maximum (°C)**	**Annual mean (mm)**
Central	22.66	17.80	28.90	1248.0 ± 284.39
Eastern	24.94	22.60	34.70	1335.7 ± 210.28
Northern	24.07	19.90	28.30	1476.0 ± 174.30
Western	21.09	19.30	26.20	921.5 ± 203.16

Table 2: ANOVA and Tukey's test (HSD output) by region.

Climate parameter	F-statistic	DF	p-value	Turkey's test	
Temperature	836.06	3	< 0.0001	Central	A
				Eastern	B
				Northern	C
				Western	D
Rainfall	41.66	3	< 0.0001	Central	B
				Eastern	B
				Northern	B
				Western	C

Table 3: Seasonal Mann-Kendall output for the different regions.

Region	Tau b	p-value	Sen's slope	Risk (%)
Central	0.244	> 0.0001	0.015	0.01
Eastern	0.027	0.4222	0.002	43.97
Northern	0.595	> 0.0001	0.058	0.01
Western	0.468	>0.0001	0.038	0.05

Table 4: Mean annual rainfall, and mean standard precipitation indices by region.

Region	Mean SPI
Central	0.1225
Eastern	0.0000
Northern	-0.0003
Western	-0.0005

Table 5: Mean Evaporation and mean Evapotranspiration by region.

Region	Evaporation (mm/year)	Calculated potential evapotranspiration (mm/month)
Central	139.0	4.97
Eastern		5.26
Northern	168.0	5.15
Western	120.8	4.78

Table 6: Monthly water requirement (inflows) in gallons per minute (gpm)/ acre for levee ponds by region.

	Region			
Month	**Northern**	**Central**	**Western**	**Eastern**
Jan	1.56	0.85	0.00	0.92
Feb	1.41	1.08	0.00	0.83
Mar	0.02	0.00	0.00	0.00
Apr	0.00	0.00	0.00	0.00
May	0.00	0.00	0.00	0.00
Jun	0.00	0.91	0.00	0.00
Jul	0.00	0.90	0.19	0.00
Aug	0.00	0.08	0.00	0.00
Sep	0.00	0.00	0.00	0.00
Oct	0.00	0.00	0.00	0.00
Nov	0.00	0.00	0.00	0.00
Dec	1.01	0.31	0.00	0.68

Table 7: Mean concentrations of the different water quality parameters measured in pond water sources by region.

	Region			
Water parameter	**Central**	**Eastern**	**Northern**	**Western**
Total hardness (mg/L)	50.3±11.40 (6.3-258.6)	89.0±16.62 (21.5-502.9)	102.7±22.49 (12.7-533.8)	66.0±13.35 (5.5-332.3)
Calcium hardness (mg/L)	20.9±4.97 (0.0-109.0)	43.3±5.94 (8.1-133.6)	34.0±5.86 (3.2-114.2)	31.1±7.38 (0.0-140.0)
Total alkalinity (mg/L)	43.2±8.99 (6.0-69.8)	80±9.16 (7.9-238.1)	106±20.72 (13.7-441.1)	55.5±12.95 (6.4-329.3)
Specific conductivity (µS/cm)	174.7±29.16 (38.4-557)	298.2±46.89 (79.0-469.0)	351.1±71.70 (53.3-1377.5)	157.6±19.52 (25.9-383.9)
pH	7.3±0.20 (5.9-10.6)	9.8±0.34 (7.0-13.0)	8.3±0.24 (5.2-13.0)	7.0±0.19 (5.3-9.4)

Table 8: Chi square test tests for water quality parameters measured by region.

Water parameters	Chi square	P-value	Cramer's V	DF
Specific conductivity	10.196	0.017	0.291	3
pH	16.498	0.000	0.371	3
Total alkalinity	14.385	0.002	0.379	3
Total hardness	8.8336	0.032	0.271	3

Table 9: ANOVA output for the different water quality parameters by region.

	ANOVA output			
Water parameter	F-statistic	DF	p-value	Turkey's test
pH	25.44	3	< 0.0001	Central C
				Eastern A
				Northern B
				Western C
Total alkalinity	4.18	3	0.0075	Central B
				Eastern A, B
				Northern A
				Western B
Total hardness	2.00	3	0.1175	Central A
				Eastern B
				Northern B
				Western A
Calcium hardness	2.28	3	0.0828	Central A
				Eastern A
				Northern A
				Western A
Specific conductivity		4.15	0.0056	Central B
				Eastern A, B
				Northern A
				Western B

Table 10: Range of concentration of different water trace metals by region.

	Region			
Element (mg/L)	**Central**	**Eastern**	**Northern**	**Western**
Al	< 0.1- 6.3	< 0.1 – 3.0	< 0.1 – 6.0	< 0.1 – 2.5
As	< 0.1	< 0.1	< 0.1	< 0.1
B	< 0.1	< 0.1 – 0.3	< 0.1	< 0.1
Ba	< 0.1- 2.2	< 0.1 – 3.1	0.1 – 1.9	< 0.1 – 3.7
Ca	1.6 – 45.5	8.9 – 117.0	4.7 – 141.0	1.9 – 103.0
Cd	< 0.1	< 0.1	< 0.1	< 0.1
Cr	< 0.1	< 0.1	< 0.1	< 0.1
Cu	< 0.1	< 0.1	< 0.1	< 0.1 – 0.7
Fe	< 0.1 – 16.7	< 0.1 – 8.2	< 0.1 – 6.5	< 0.1 – 11.9
K	0.4 – 10.0	0.9 – 43.6	0.9 – 26.8	0.3 – 9.0
Mg	0.7 – 58.2	4.0 – 104.0	2.3 – 80.0	1.2 – 43.7
Mn	< 0.1 – 2.3	< 0.1 – 2.2	< 0.1 – 1.2	< 0.1 – 1.5
Na	8.3 – 52.9	12.8 – 77.5	7.7 – 136.0	8.9 – 68.3
Ni	< 0.1	< 0.1	< 0.1	< 0.1
P	< 0.1 – 1.7	< 0.1 – 2.0	< 0.1 – 1.2	< 0.1 – 2.2
Pb	< 0.1	< 0.1	< 0.1	< 0.1
Zn	< 0.1 – 0.2	< 0.1	< 0.1	< 0.1

Table 11: Percentage distribution of pond water sources in terms of iron and aluminum concentrations by region.

	Iron (%)		Aluminum (%)	
Region	**Optimal**	**Non-optimal**	**Optimal**	**Non-optimal**
Central	53.3	46.7	40	60
Eastern	66.7	33.3	40	60
Northern	70.0	30.0	53	47
Western	36.7	63.3	70	30

Table 12: Suitability ratings of different water bodies for cage culture by small to medium sized facilities for five Uganda lakes.

	Lake				
Water parameter	**Victoria**	**Albert**	**Edward**	**George**	**Kyoga**
Water depth	3	3	3	1	1
Secchi depth	3	2	2	1	2
Dissolved oxygen profile	3	2	2	1	1
Long axis to the bay	3	3	2	1	2
Current between the bay and the lake	3	3	2	1	2
Connection of the bay to open water of the lake	3	2	1	1	1
Distance to obvious source of pollution	3	3	2	1	1
Total score	21	18	14	7	10

Table 13: Mean water quality parameters for five Uganda lakes.

	Lake				
Parameter	**Victoria**	**Albert**	**Edward**	**George**	**Kyoga**
Depth (m)	10.80	20.90	13.70	1.60	2.70
Secchi depth (m)	2.90	1.90	1.40	0.30	1.20
Temperature (°C)	25.50 ± 0.03	27.90 ± 0.04	26.40 ± 0.06	27.20 ± 0.11	28.10 ± 0.18
Total dissolved solids	0.06 ± 0.00	0.42 ± 0.00	0.58 ± 0.00	0.32 ± 0.03	0.16 ± 0.01
(mg/L) Salinity (PSU)	0.05 ± 0.00	0.31 ± 0.00	0.44 ± 0.00	0.24 ± 0.02	0.12 ± 0.01
Dissolved oxygen (mg/L)	6.64 ± 0.14	5.31 ± 0.08	5.41 ± 0.40	2.77 ± 1.50	2.76 ± 0.56
Specific conductivity (µS/cm)	97.70 ± 0.06	640.80 ± 2.20	886.40 ± 2.16	485.40 ± 48.77	252.60 ± 17.80
pH	8.40 ± 0.05	8.80 ± 0.07	9.30 ± 0.06	7.90 ± 0.50	7.40 ± 0.16
Total alkalinity (mg/L)	38.65 ± 0.11	265.52 ± 2.78	382.01 ± 0.08	102.36 ± 1.07	43.58 ± 2.04
Total hardness (mg/L)	24.16 ± 0.74	140.32 ± 1.06	215.22 ± 0.08	86.72 ± 1.00	28.06 ± 1.27
Calcium hardness (mg/L)	5.49 ± 1.66	22.36 ± 1.36	26.49 ± 0.05	39.46 ± 0.04	8.92 ± 2.29

Table 14: Mean concentrations of the different soil quality parameters by region.

Soil parameter	Region			
	Central	Eastern	Northern	Western
Soil pH	5.3±0.11 (4.3-6.6)	6.3±0.16 (4.7-7.8)	6.4±0.19 (4.5-8.3)	5.5±0.14 (4.6-7.4)
% Total Nitrogen	0.1±0.01 (0.0-0.2)	0.2±0.02 (0.0-0.5)	0.1±0.01 (0.0-0.3)	0.5±0.10 (0.0-2.1)
% Total Carbon	1.2±0.13 (0.2-2.4)	1.7±0.21 (0.4-4.5)	1.2±0.11 (0.1-3.0)	6.4±1.38 (0.5-30.5)
% Organic matter	2.1±0.23 (0.4-4.2)	2.9±0.37 (0.6-7.7)	2.0±0.18 (0.2-5.2)	10.9±2.38 (0.9-52.5)
Saturated hydraulic conductivity (cm/hr)	0.6±0.07 (0.2-1.3)	0.5±0.08 (0.2-1.6)	0.4±0.05 (0.1-1.1)	0.8±0.13 (0.1-1.3)

Table 15: Chi square test output for the different soil quality parameters in the different regions.

Soil parameters	Chi square	P-value	Cramer's V	DF
Soil pH	20.508	0.0001	0.413	3
Total Carbon (%)	16.705	0.0008	0.373	3
Organic matter (%)	12.103	0.0070	0.318	3

Table 16: ANOVA output for the different soil quality parameters analyzed by region.

Soil parameter	ANOVA output				
	F-statistic	DF	p-value	Turkey's test	
Soil pH	13.54	3	< 0.0001	Central	B
				Eastern	A
				Northern	A
				Western	B
Total Carbon(%)	12.64	3	< 0.0001	Central	B
				Eastern	B
				Northern	B
				Western	A
Total Nitrogen (%)	14.08	3	< 0.0001	Central	B
				Eastern	B
				Northern	B
				Western	A
Organic matter (%)	12.63	3	< 0.0001	Central	B
				Eastern	B
				Northern	B
				Western	A
Saturated hydraulic conductivity (cm/hr)	3.17	3	0.0269	Central	B
				Eastern	B
				Northern	A,BA
				Western	

Table 17: Trace metal concentration ranges by region.

Element (mg/L)	Region			
	Central	**Eastern**	**Northern**	**Western**
Al	18.0 – 295.0	19.0 – 350.0	39.0 – 903.0	3.0 – 1153.0
As	< 0.1	< 0.1	< 0.1	< 0.1
B	0.3 – 1.7	0.3 – 2.3	0.3 – 2.7	0.4 – 2.1
Ba	1.4 – 11.2	2.0 – 10.4	0.8 – 7.4	1.2 – 13.0
Ca	104.0 – 1930.0	311.0 – 3299.0	527.0 – 3161.0	457.0 – 3146.0
Cd	< 0.1	< 0.1	< 0.1	< 0.1
Cr	< 0.1	< 0.1	< 0.1	< 0.1
Cu	0.7 – 3.7	0.2 – 11.5	0.3 – 11.5	0.0 – 4.8
Fe	51.0 – 470.0	6.0 – 415.0	7.0 – 847.0	3.0 – 591.0
K	18.0 – 136.0	26.0 – 160.0	18.0 – 209.0	26.0 – 126.0
Mg	40.0 – 596.0	100.0 – 715.0	112.0 – 667.0	123.0 – 1132.0
Mn	10.0 – 279.0	3.0 – 295.0	8.0 – 225.0	4.0 – 336.0
Na	38.0 – 184.0	45.0 – 192.0	55.0 – 298.0	40.0 – 324.0
Ni	< 0.1	< 0.1	< 0.1	< 0.1
P	< 0.1 – 22.0	< 0.1 – 62.0	< 0.1 – 26.0	< 0.1 – 372.0
Pb	< 0.1	< 0.1	< 0.1	< 0.1
Zn	0.6 – 16.5	0.5 – 7.4	0.7 – 14.0	0.2 – 16.0

Table 18: Liming requirement in the different regions.

Lime requirement	Region						
	Central		**Eastern**		**Northern**	**Western**	
(kg/ha)	1420 ± 259.79	(91 – 4086)	749 ± 151.44	(126 – 1512)	829 ± 274.42	(91 – 3528)	2970 ± 414.42	(272– 5400)
(per average pond)	355 ± 64.95	(23 – 1022)	187 ± 37.86	(32 – 378)	207 ± 68.60	(23 – 882)	743 ± 103.61	(68 – 1350)

Table 19: Liming materials with their liming properties and prices.

Type of liming material	Neutralizing value (NV)	Fineness value (FV)*	Effective neutralizing value (ENV)	Price	Price of effective lime)
	(%)	(%)	(%)	($/ton)	($/ton)
Grey lime	22.0	46.1	10.1	302	2980
Neelkanth Ltd hydrated lime class A	51.8		51.8	504	973
Stock feed agricultural lime	35.6	25.0	8.9	252	2833
Tororo hydrated lime	38.7		38.7	302	780

Table 20: Liming rate by region using Tororo hydrated lime.

	Region			
Liming rates	**Central**	**Eastern**	**Northern**	**Western**
Lime requirement (kg/ha)	2741	1446	1600	5734
Lime rate (per average pond)	685	362	400	1433

Table 21: Observed seepage and calculated seepage in the different regions.

	Region			
Seepage rates	**Central**	**Eastern**	**Northern**	**Western**
Observed seepage (cm/day)	3.37 ± 2.03	2.27 ± 0.39	2.53 ± 1.41	0.93 ± 0.17
Calculated seepage (cm/day)	14.14 ± 1.67	12.23 ± 1.84	9.29 ± 1.12	16.06 ± 1.79

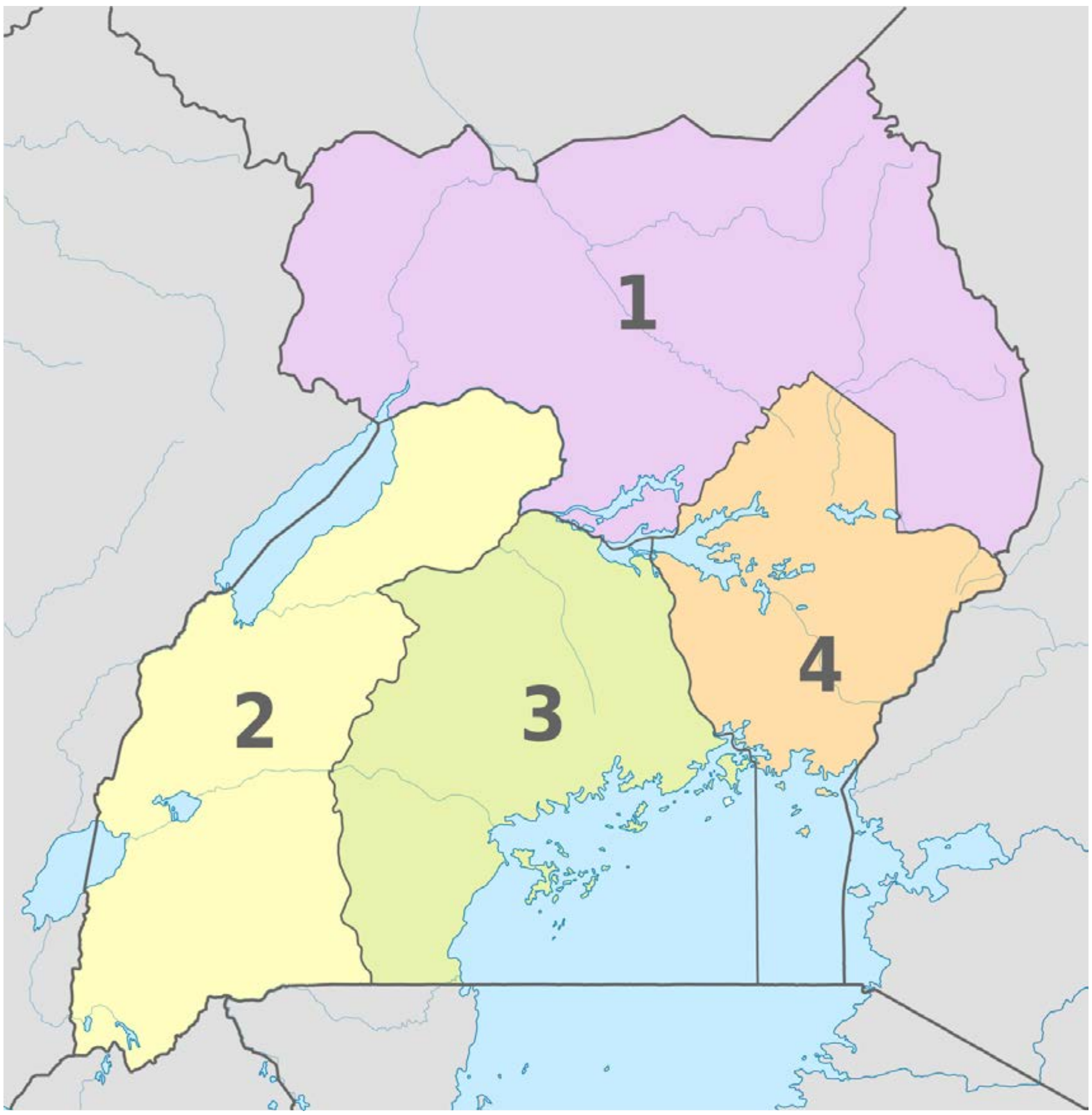

Figure 1: Administrative map of Uganda showing Northern region (1), Western region (2), Central region (3) and Eastern region (4) (TUBS, 2012).

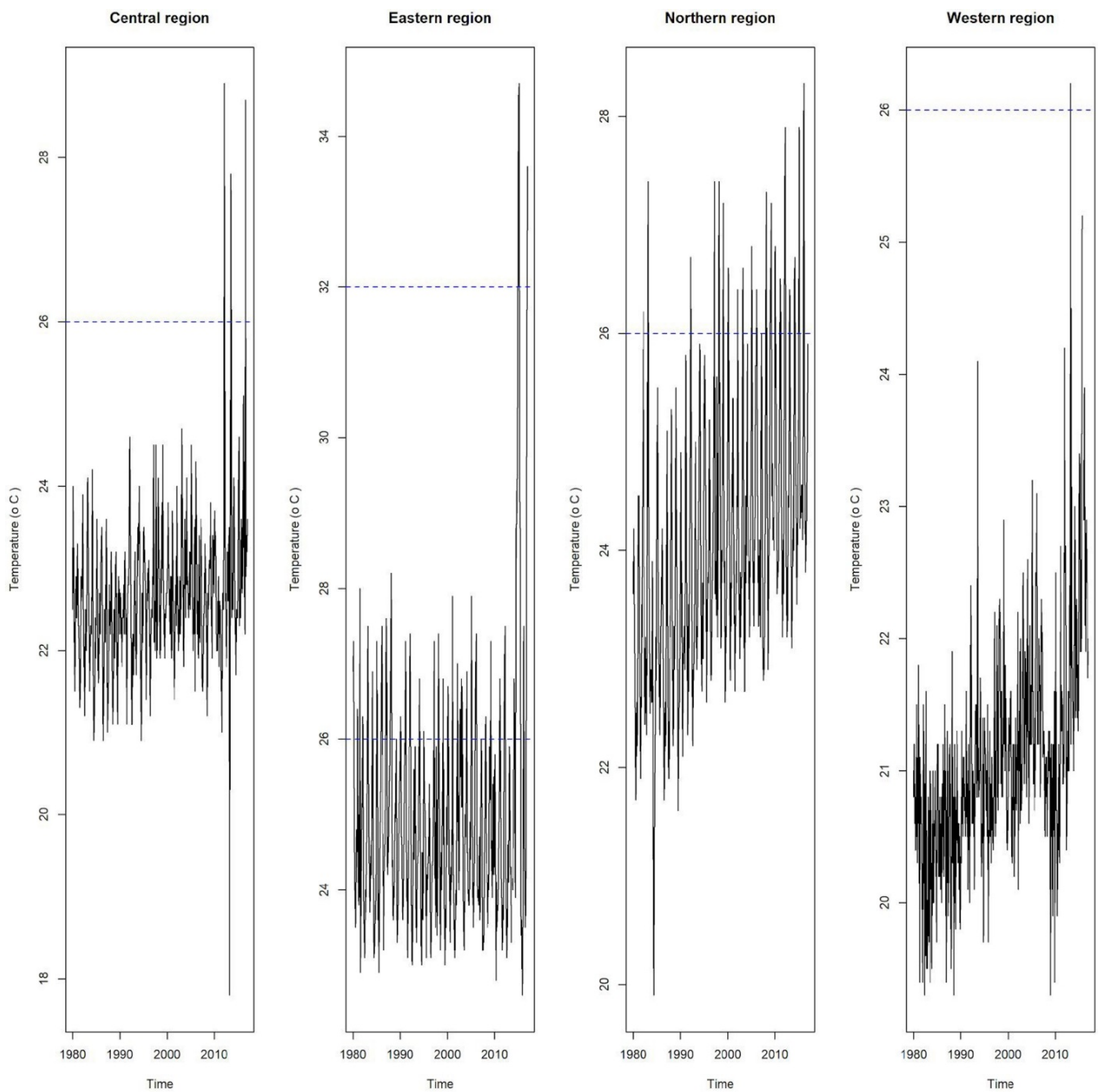

Figure 2: Mean Temperature for different regions for period 1980-2016.

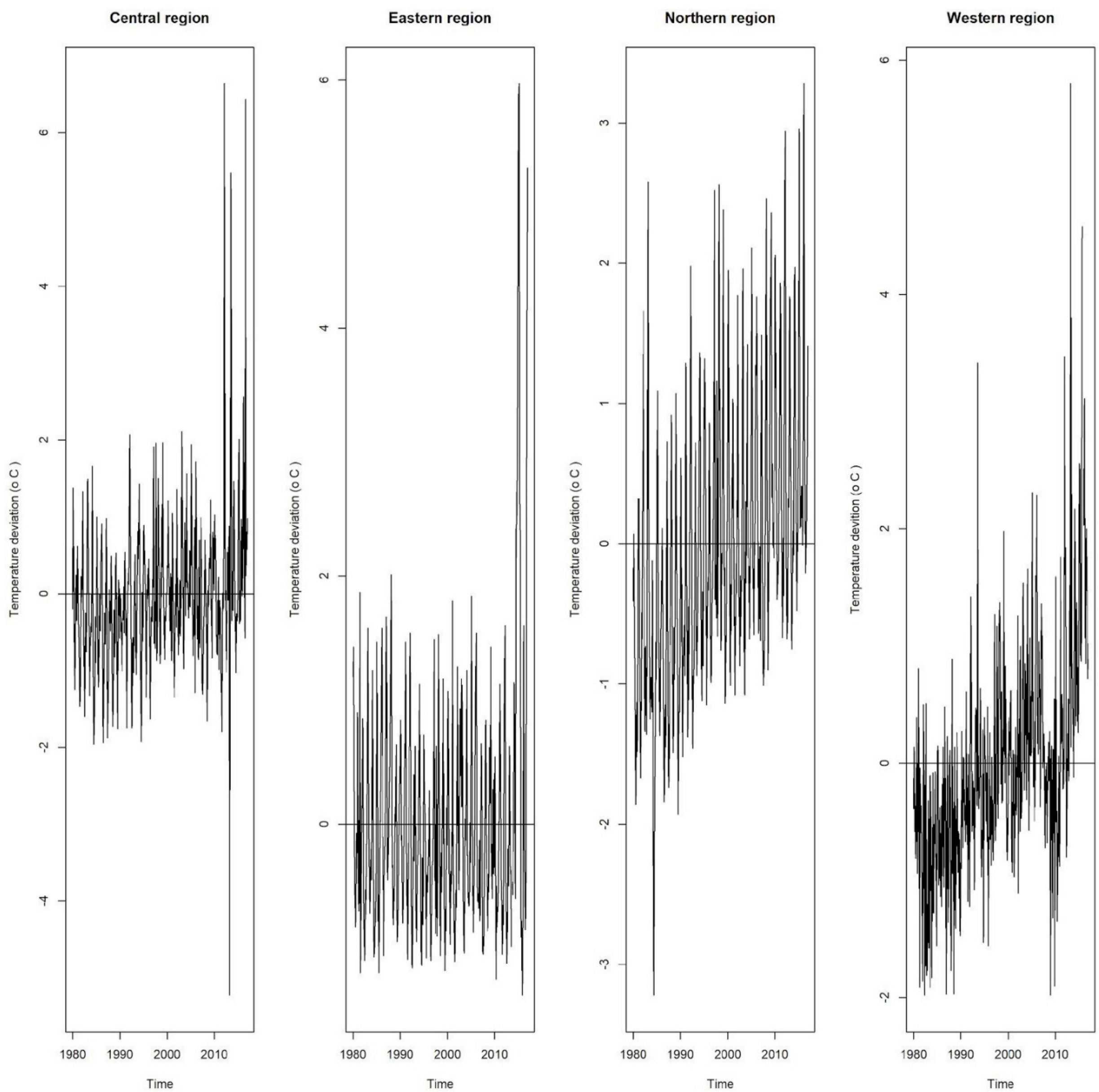

Figure 3: Temperature deviations from the long term mean for the different regions for a period 1980-2016.

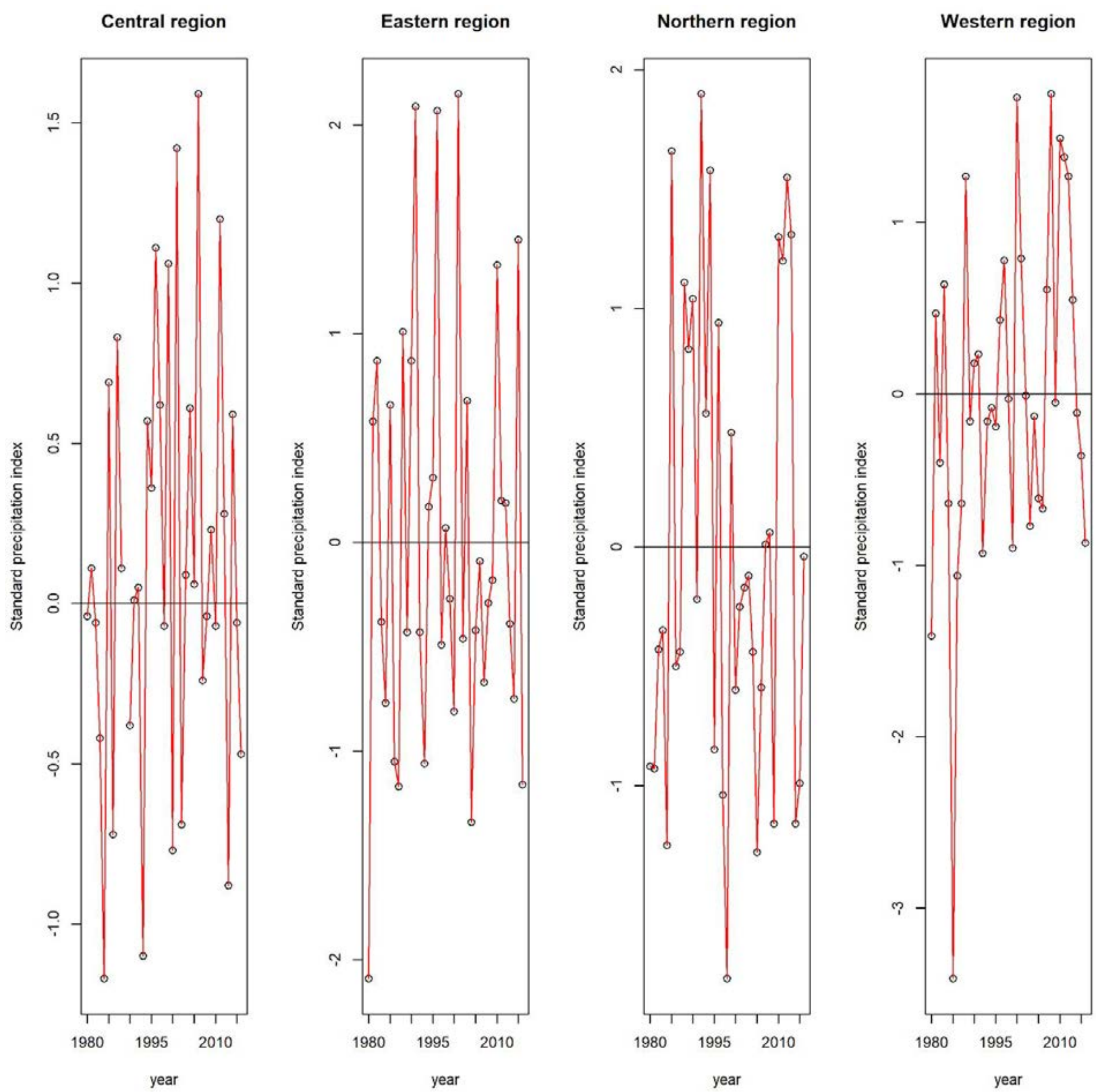

Figure 4: 12-month Standard precipitation index for the different regions for a period of 1980- 2016.

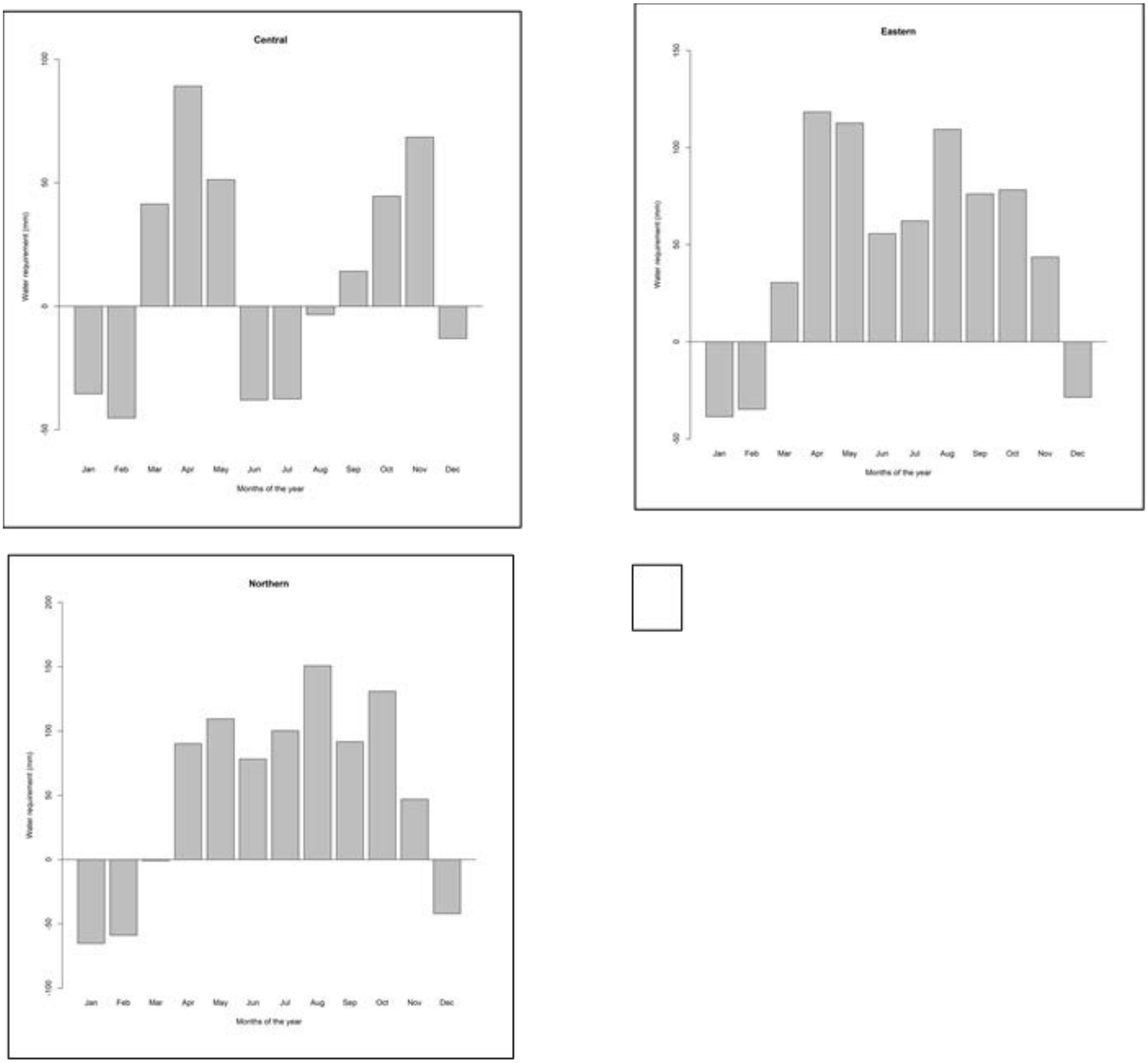

Figure 5: Monthly water requirement for the different regions

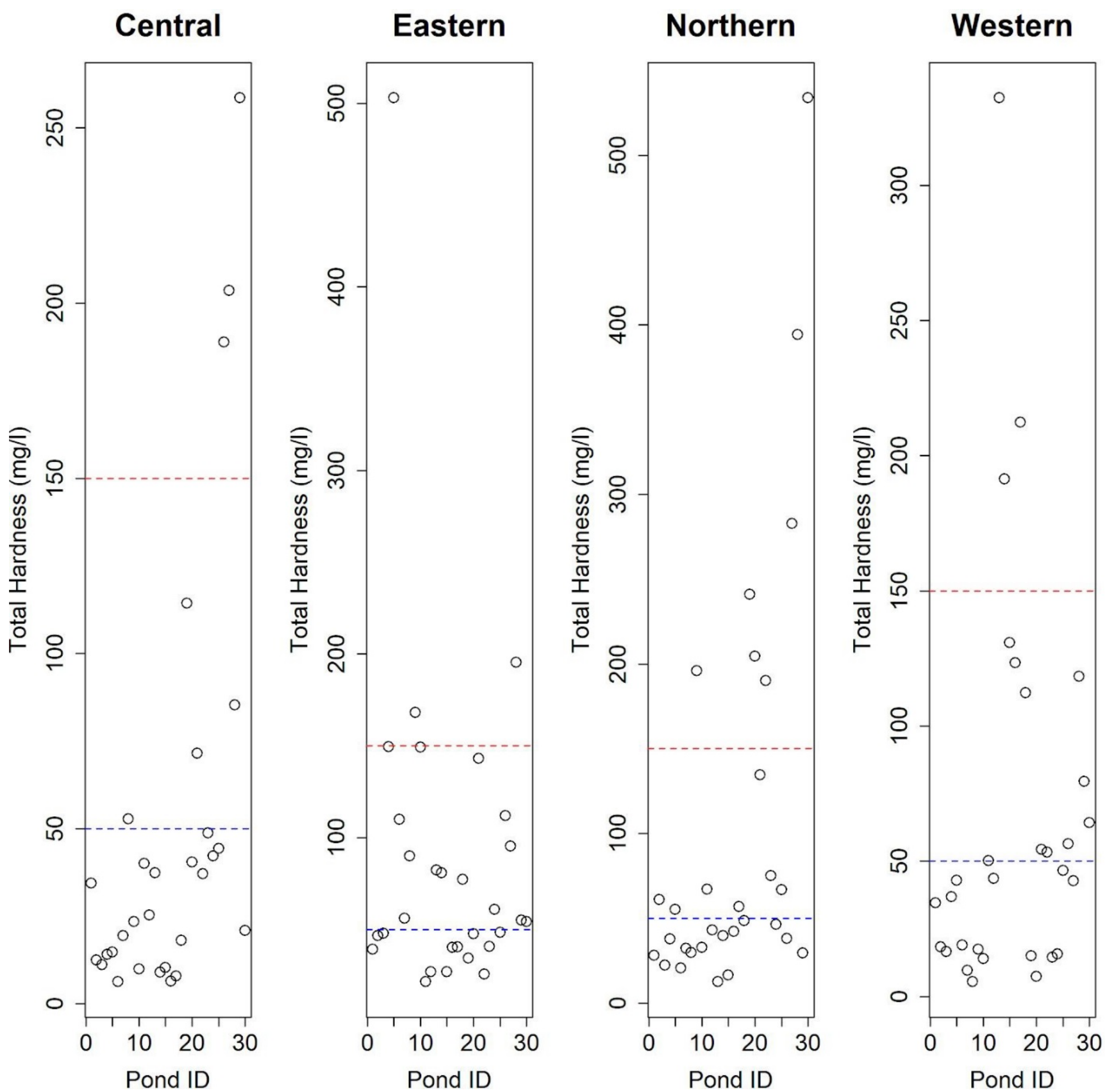

Figure 6: Frequency distribution of different pond water sources for total hardness concentration.

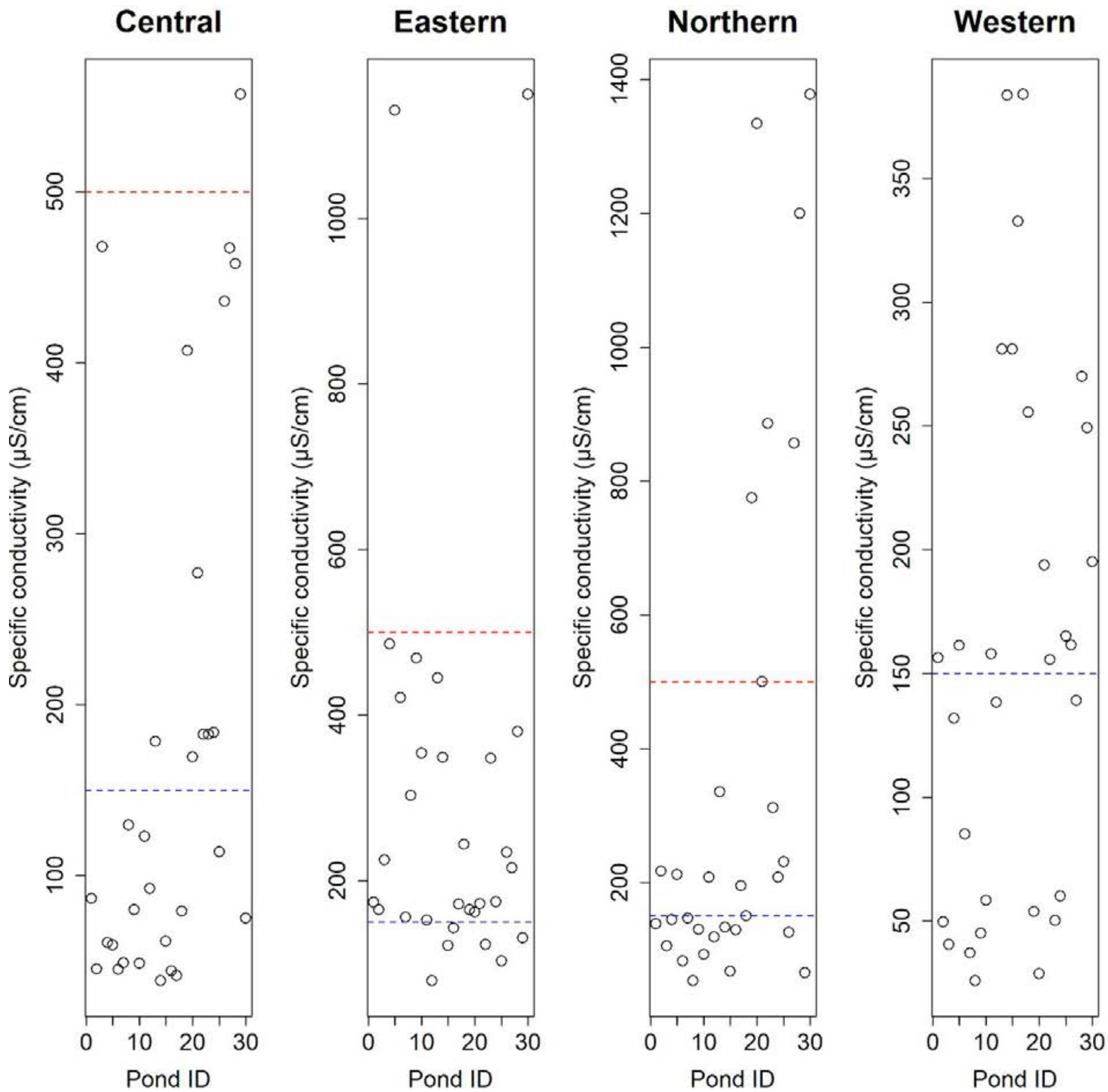

Figure 7: Frequency distribution of different pond water sources for specific conductivity concentration.

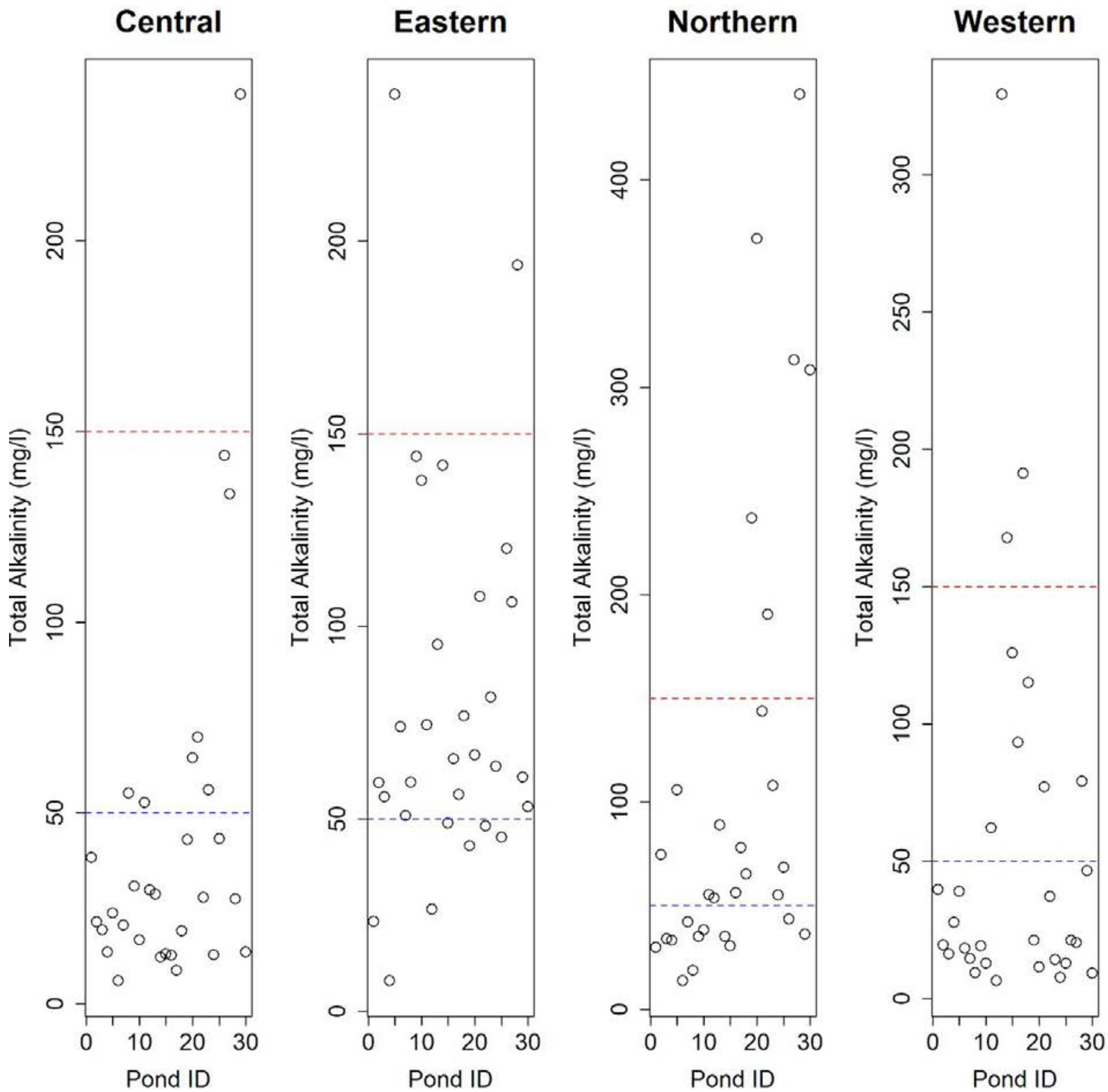

Figure 8: Frequency distribution of different pond water sources for total alkalinity concentration.

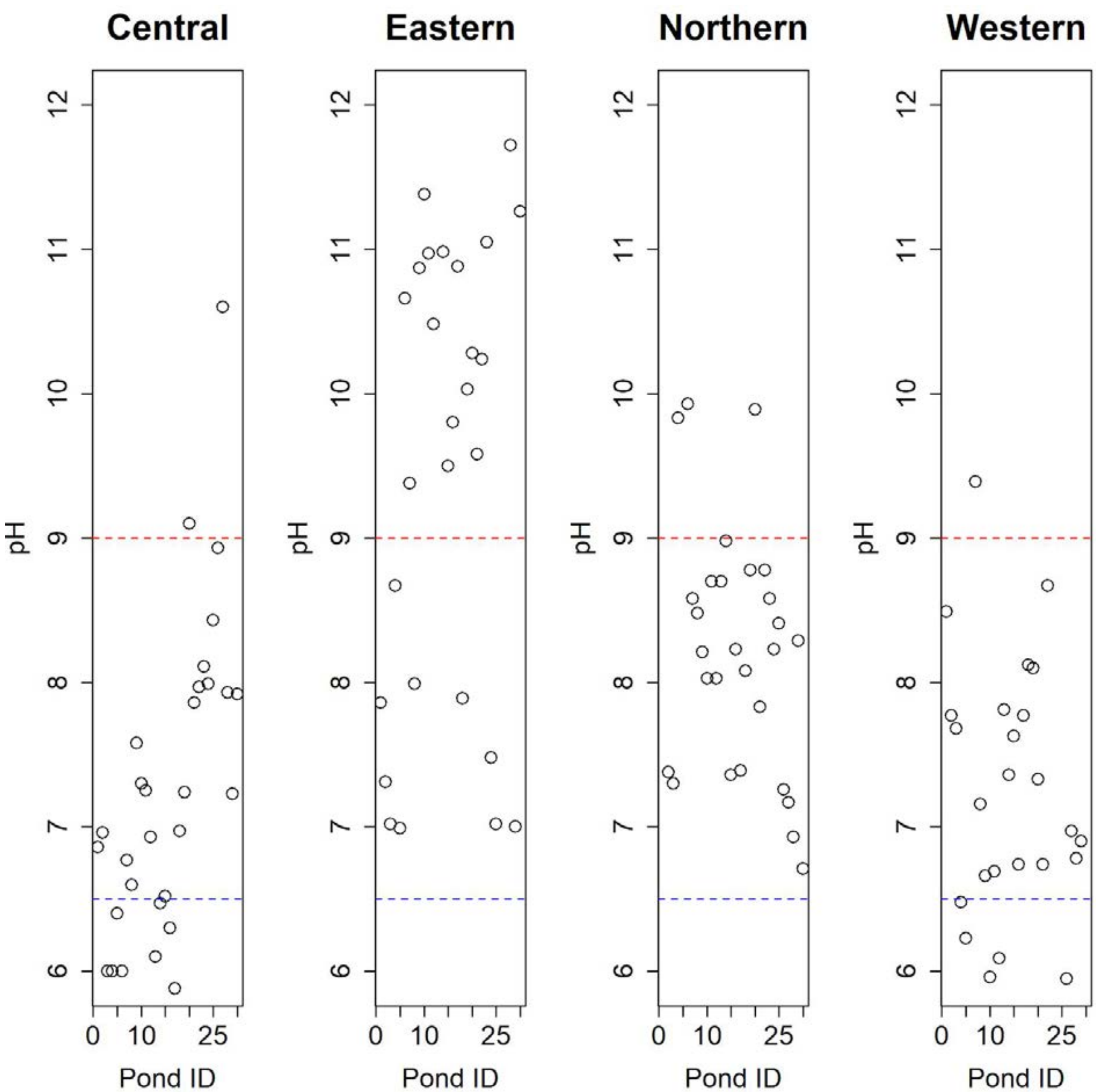

Figure 9: Frequency distribution of different pond water sources for pH concentration.

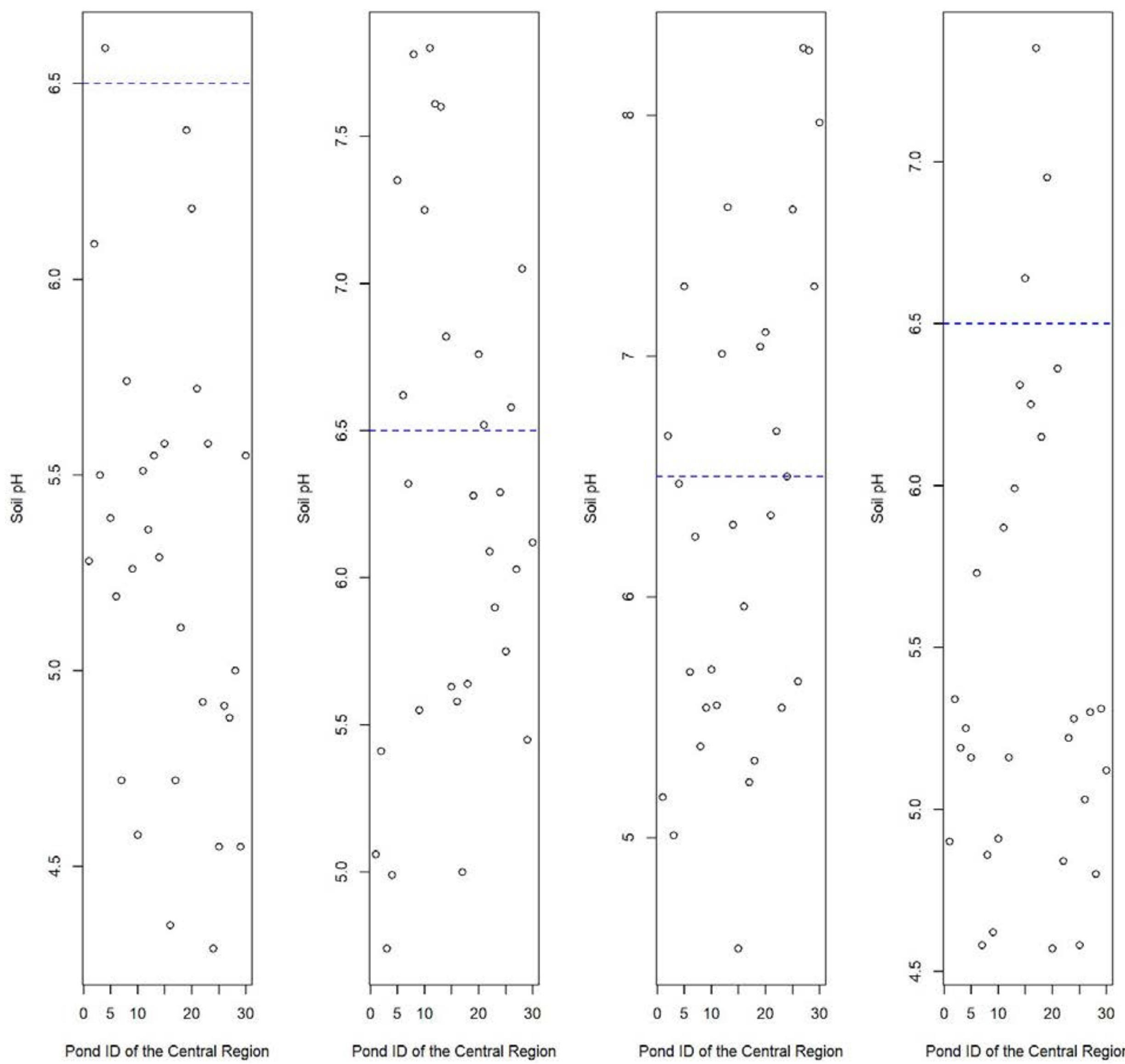

Figure 10: Frequency distribution of different pond water sources for soil pH.

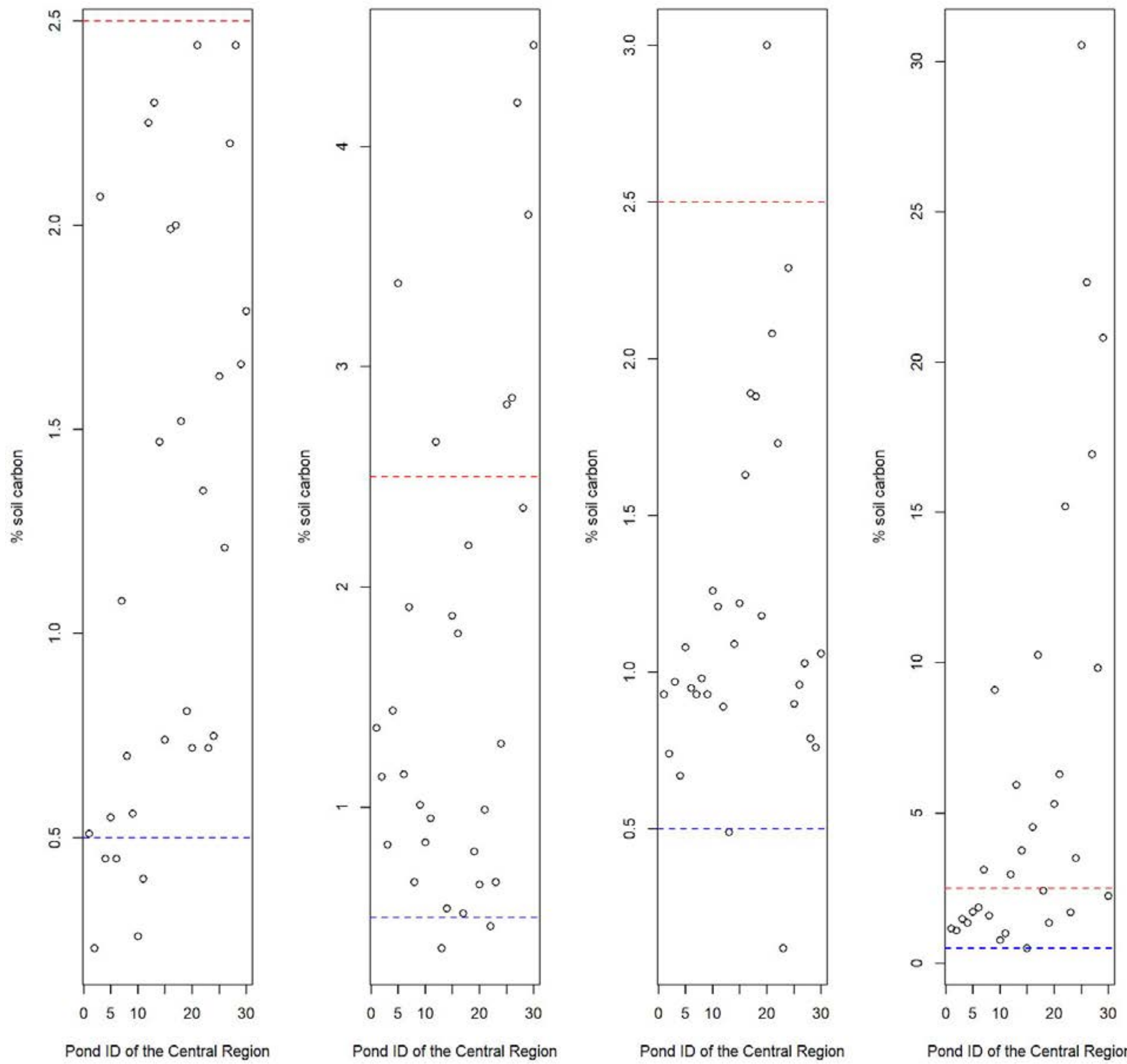

Figure 11: Frequency distribution of different pond water sources for total carbon.

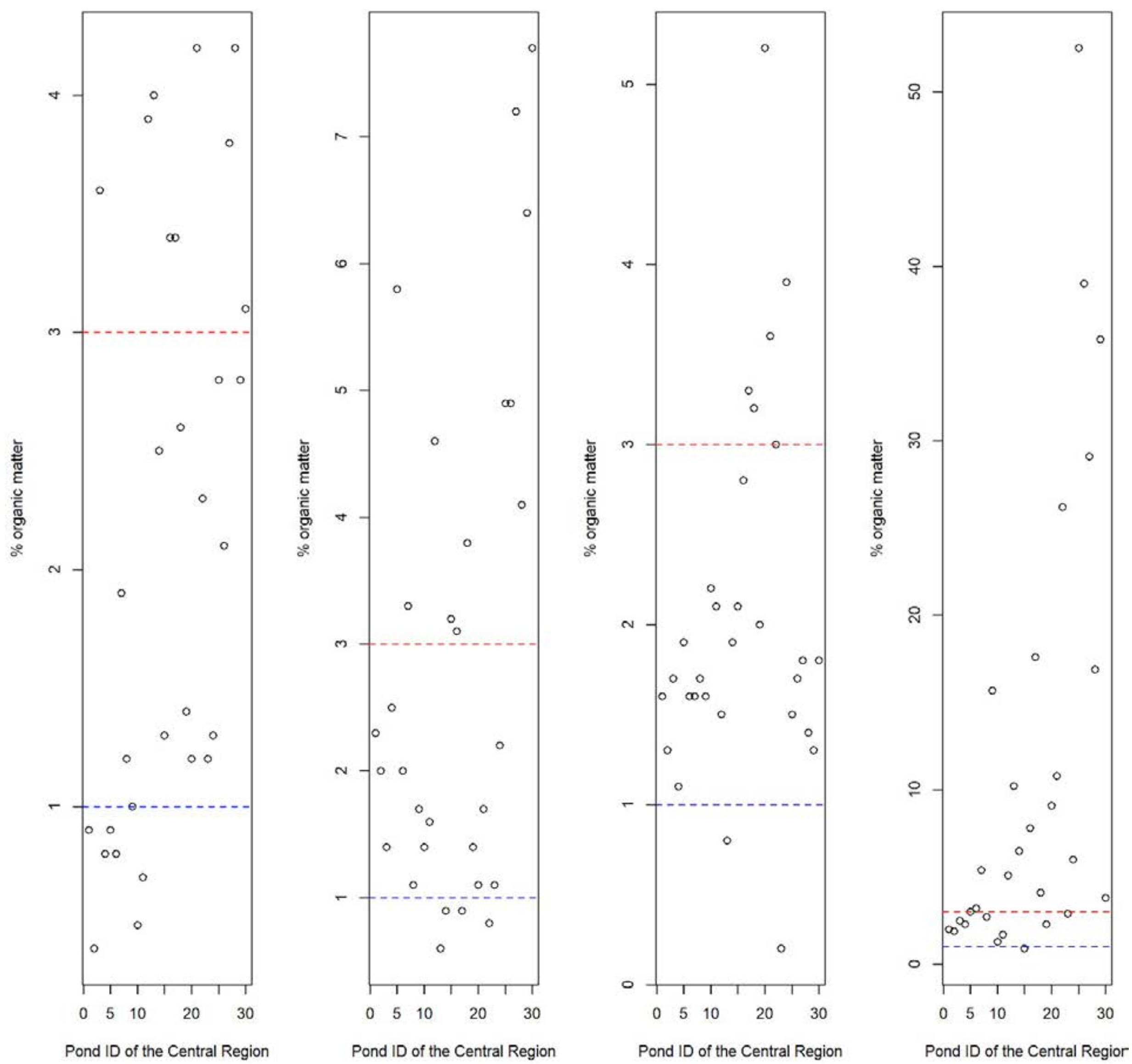

Figure 12: Frequency distribution of different pond water sources for organic matter.

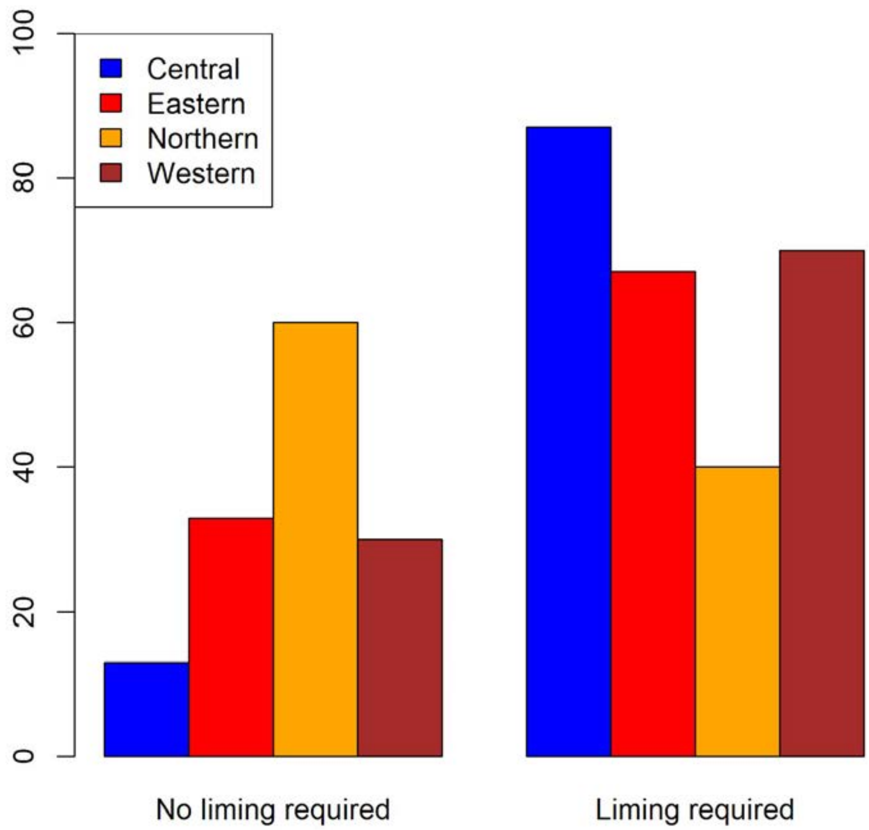

Figure 13: Percentage frequency of the ponds that needed liming and those that did not in the different regions.

ADDITIONAL INFORMATION

Water Quality Management Recommendations for Tilapia and African Catfish Culture in Uganda-A list of water quality management procedures for use in pond culture of tilapia and African catfish are provided below:

Tilapia

1. The water source for the pond such as a well or stream or water from the pond that is filled by runoff should be analyzed. The minimum analysis would include; pH, total alkalinity, and electric conductivity. A complete analysis including trace elements would be desirable for a better understanding of the water quality. However, a survey of water sources and ponds suggested that low pH and alkalinity were the usual problems.
2. The area around the water source and watershed of the pond should be examined for sources of erosion that could create turbidity in the ponds or for pollution that could be harmful to fish. Highly polluted sites should be neglected for building new ponds. Erosion on watersheds usually can be prevented by establishing grass cover.
3. Many ponds in Uganda have acidic bottom soils and lower alkalinity than necessary for good fish production. Although this limitation is easily corrected by liming, there does not seem to be a source of good quality liming material. The best product located (Neelkanth Ltd hydrated lime) had an effective neutralizing value of only 50% of best quality agricultural limestone.
4. Hydrated lime at applications above 100 kg/ha of high quality product will raise pond pH enough to sometimes kill fish. Thus, the hydrated lime product would have to be applied to the pond bottom between crops when there are no fish in the pond. The pond should be allowed to stand after refilling until the pH is below 8.5. This usually will take about 2-3 weeks, but if the pond owner has a pH meter or kit, the pH can be measured and possibly allow less fallow time.

5. The recommended rates for the use of the Neelkanth Ltd hydrated lime for ponds of different alkalinities follow:

Alkalinity (mg/L)	Neelkanth Ltd hydrated lime (kg/ha)
Below 10	8,000
10-20	6,000
20-40	4,000
Above 40	0

6. Farmers should urge the government to either establish some standards for liming materials or import liming materials of better quality than the domestic products. Note: Possibly there are other sources of liming materials of better quality that were overlooked in the study.
7. If pH is below 4.5 in pond water, there will be no alkalinity. The best option usually is to abandon such sites. But, if desired, high rates of liming can be tried.
8. Liming material should be spread uniformly over ponds. It should be applied at least 2 weeks before phosphate fertilizer is applied to ponds. Liming material can be applied before, at the same time, or soon after the first application of organic fertilizer.
9. Alkalinity should be measured annually in ponds and liming materials re-applied in accordance with measured alkalinity (see #5).
10. In ponds where feed will not be applied, fertilizers are necessary to increase fish production. Organic fertilizers such as fresh-cut grass, leaves, livestock manure, or chicken manure may be used. Alternatively, chemical fertilizers such as urea and triple superphosphate may be used to fertilize ponds.
11. It is difficult to establish the maximum safe amount of organic fertilizers for a pond, because organic fertilizers vary in water and nutrient content. The maximum rate probably should not exceed 200 kg/ha/day for grasses, hay and leaves or 100 kg/ha/day for animal manure. Of course, organic fertilizers may be applied daily, every other day, three times weekly, or even weekly. But, organic matter uses oxygen as it decomposes. Warning: Excessive organic fertilizer application can lead to dissolved oxygen depletion and fish kills may result.
12. Chemical fertilizers have a known nutrient content making the application rate easier to determine. The most common chemical fertilizers used in ponds are urea and triple super phosphate. Satisfactory application rates usually are 15 – 20 kg/ha triple superphosphate.
13. Fertilizers should be applied at 1- 2 weeks' intervals until a good phytoplankton bloom is established. Afterwards, fertilizers should be applied as necessary to maintain the phytoplankton bloom.
14. The abundance of phytoplankton typically is gauged by water clarity. In a properly fertilized pond for tilapia, underwater visibility usually will be around 30 – 40 cm.
15. The underwater visibility can be checked with a ruler or other measuring stick to which a white object attached at the end. The measuring device is extended vertically downward into the water until it first disappears from sight. The depth of underwater visibility is read from the ruler at the water surface. Note: It is possible to purchase a Secchi disk for measuring water clarity. This device is a weighted 20-cm diameter disk with calibrated line attached. It is lowered into the water and the depth at which it disappears is recorded.
16. Fertilizers should be re-applied when the underwater visibility is less than 45 cm. Do not wait until the water clears more before making another fertilizer application. A high abundance of phytoplankton must be maintained to support fish production.
17. Another reason for maintaining a good phytoplankton bloom is for underwater weed control. In clean ponds, dense infestations of underwater plants that are undesirable in fish ponds may grow profusely.
18. The fertilizers for applying in a pond should be weighed and placed in a large pail. The pail should be filled with water and 15 – 30 minutes allowed for fertilizers to dissolve. The water and fertilizer should be stirred vigorously for 2 – 3 minutes after which the mixture should be splashed over the pond surface.
19. Dense phytoplankton bloom (underwater visibility less than 10 – 15 cm) should be avoided because too much phytoplankton may result in nighttime dissolved oxygen depletion and fish mortality.
20. Farmers may choose to feed tilapia in ponds rather than to fertilize ponds to increase tilapia production.

21. In ponds with feeding, it still is desirable to apply agricultural limestone to ponds with low alkalinity water.
22. Pelleted feed should be applied one or two times daily. Usually, it is more effective to fish growth the daily feed allowance is offered in two applications rather than a single application.
23. The feed application rate should be gradually increased as the fish grows. Usually, early in the grow-out period, feed is applied at 3- 4% of the estimated weight of fish in the pond. Later, the feeding rate may be reduced to 2.5% or even 2 % of fish weight in ponds.
24. Water from feeding enrich ponds with nutrients leading to phytoplankton blooms. Too much phytoplankton can cause dissolved oxygen depletion especially at night.
25. Tilapia are hardy and withstand low dissolved oxygen concentration well. But, low dissolved oxygen concentration can kill them.
26. In order to minimize the possibility of fish kills from oxygen depletion, daily feed input probably should not exceed 75 kg/ha/day, but to be safe, a limit of 50 kg/ha/day is prudent.
27. Ammonia can be toxic to fish, but if dissolved oxygen concentration is adequate in ponds with feeding, ammonia toxicity to tilapia is seldom seen.
28. Some fish farmers apply bacterial products (usually living cultures of bacteria) often called probiotics to ponds for removing ammonia and improving other aspects of water quality. There is no evidence from research that probiotics are effective.
29. Water exchange may be used in pond with feeding to increase the dissolved oxygen supply and allow greater feed input and fish production. Rates of water exchange from 25% to several hundred percentage of pond volume per day have been used, but there is no reliable method for determining the acceptable feeding rate at a water exchange rate other than by monitoring the dissolved oxygen concentration.
30. Another way to increase fish production in ponds with feeding is to apply mechanical aeration. The relationship between aeration rate and fish production is well established.
31. Several kinds of aerators are available, but paddlewheel aerators are most commonly used in fish ponds. Both electric and diesel-powered devices are available.
32. The general “rule of thumb” for aeration is to install one horse power (hp) of aeration for 500 kg of fish above 2,000 kg/ha. For example, to produce 5,000 kg/ha of fish, the calculation of aeration requirement follows;
33. 5,000 kg fish/ha - 2,000 kg fish/ha = 3000 kg fish/ha
34. 3,000 kg fish/ha/500 kg fish/ha/hp = 6 hp aerators/ha
35. Aerators should be placed in water of 0.75 m or more in depth. When multiple aerators are installed in a pond, they usually are positioned to cause a circular water flow pattern.
36. Ponds usually have high dissolved oxygen concentration during the day, and aerators can be turned off. The critical period for aeration in fish ponds typically is at night.
37. Ammonia often accumulates in pond with feeding and aeration because of large feed input. Ammonia is toxic to fish, but tilapia are quite tolerant to ammonia. Unless total ammonia nitrogen concentrations consistently are 5 – 10 mg/l, ammonia stress or toxicity would not be expected unless pH in pond water was above 9. Test kits can be purchased for measuring pH and ammonia, but ammonia and pH monitoring in tilapia ponds is not usually necessary.
38. The only effective way of reducing ammonia concentrations in ponds (aside from lowering feed inputs which is not desirable and does not provide immediate ammonia reduction) is to flush water through ponds.

African Catfish

39. This air-breathing species is very tolerant to low dissolved oxygen and poor water quality conditions. As a result, little water quality management is necessary.
40. The only recommendation is to apply liming materials to acidic, low alkalinity ponds. The instructions for liming tilapia ponds may be followed.

COASTAL WOMEN'S SHELLFISH AQUACULTURE DEVELOPMENT WORKSHOP

Production System Design and Best Management Alternatives /Experiment/13BMA01PU

Narriman Jiddawi[1], Maria Haws[2], and Kwamena Quagrainie[3]

[1]*Institute of Marine Sciences (IMS), University of Dar es Salaam, Zanzibar, Tanzania*
[2]*Pacific Aquaculture and Coastal Resources Center, University of Hawaii-Hilo*
[3]*Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA*

ABSTRACT

Women play various roles along the coast of, Zanzibar which contribute to their socio-economic wellbeing. Women have traditionally been involved in gleaning the coast, collecting shells of different types, collecting sea cucumber, octopus, and crab harvesting, and recently seaweed farming. Of late they are also actively involved in processing and selling fish, in bivalve farming from 2003, and in pearl farming from 2006, including jewelry making using shells. Despite their involvement in activities of this sector, women's operations are often small-scale and their incomes small as compared to their men counterparts. They are also faced with various constraints. The aim of the activity was to empower women with knowledge to help them improve their activities, empower them economically through the culture of bivalves for half pearl production and making jewelry using shells in a sustainable manner through spat collectors (Crawford et al., 2010). Information is also presented on how the women have become stronger though collaborative efforts in group formation and learning of marketing and leadership skills.

INTRODUCTION

Poverty is one of the main problems facing people living in the coastal areas of Tanzania. Women occupy a strategic position in the food production processes along the coastal community. They work for long hours between 16-18 as they bear the moral obligation of doing all domestic chores in addition to their other economic activities (De La Torre-Castro and Jiddawi 2005). With the establishment of sustainable half-pearl farming and jewelry making linked with conservation, new technique for spat collection required training and extension to encourage participation.

Women have been leaders in coastal aquaculture in East Africa being the first to culture seaweed, which has become the major type of coastal aquaculture. The shellfish farming development efforts, started eight years ago were modeled on the seaweed farming efforts, both of which were supported by the research and extension efforts of IMS and WIOMSA. Shellfish farming was a natural activity for women to adopt since they were already familiar with many bivalve species due to their reef-gleaning activities. Initially. women began shellfish farming in a low intensity fashion by placing smaller specimens of the bivalves they collected from reef areas in small, fenced-in enclosures in the intertidal area to allow them to grow to eating size. The purpose of the "fences", made of short stakes, was primarily used to designate the area claimed by the women farmers and to prevent other intertidal users from treading on the enclosed bivalves. Subsequently, they found that the stakes provided good substrates for spat collection, as high numbers of spat naturally attached to the stakes. These specimens were added to the "farmed" bivalves in the enclosed area. Women typically gather, consume, and sell nearly every bivalve species (*Ostrea spp., Crassostrea spp., Isognomen spp., Donax spp., Anadara spp.*) found on the intertidal flats including two species of pearl oysters, *Pinctada margaritifera* and *Pteria penguin* (de la Torre-Castro et al., 2017, Jiddawi, 2012). The latter two species have been utilized for half-pearl production on a limited basis on Zanzibar and Pemba Islands. Hence, unlike single species industries, shellfish farmers in Zanzibar have the luxury of being able to utilize nearly all bivalve species collected on any spat collector. Of course, developing methods which would optimize collection of the higher value species such as oysters and pearl oysters would be most advantageous.

OBJECTIVES

The main objective of this study was conduct workshops that bring together technical assistance providers with participants from coastal villages. As the primary participants in shellfish farming, the majority of the participants were women and girls.

1) Provide training in bivalve shellfish culture methods;
2) Share results of the participatory research conducted as part of Investigation 1 and previous research; and
3) Develop an action plan for further development of the small-scale bivalve industry.

METHODOLOGY

The technical focus for the workshop were on spat collection, the improved nursery methods, and marketing skills. The workshops were mostly participatory in nature, and included site visits and hands-on training were also done. Workshop participants included community members, government agency representatives, village heads, district fisheries officers, private sector representatives, journalists, and local NGO's. The target for gender balance for the workshop was 50:50. Although most of the people participating in the farming activities were women, male community members also participated and provided other forms of support. Technical assistance providers were both gender. While workshops primarily focused on women shellfish farmers, the inclusion of both genders was important to achieve the expected results in developing spat collection and shellfish farming.

Several workshops were done but two major ones were held. Furthermore, since climate change impacts have already affected the other primary mariculture activity in East Africa (seaweed farming), discussions for adaptation to climate change impacts were done.

Significance

This workshop provided opportunities to provide training and a venue to discuss research and training efforts. Moreover, they brought stakeholders together to develop an action plan to guide future efforts.

RESULTS AND DISCUSSIONS

Several workshops and activities were held as listed below:

Coastal Women's Shellfish Aquaculture Development Workshop
12th August, 2017 Fumba, Zanzibar

The AquaFish project in Zanzibar hosted a one day workshop in Fumba, Zanzibar on 12th August,2017 brought together about 35 participants, of which 28 were women participant and 5 men. The participants were from villages involved in half pearl farming jewelry making using shells (Nyamanzi, Fumba Bondeni, Fumba Chaleni, Bweleo, Unguja Ukuu and Kizingo) and conducting mariculture activities (seaweed and seacucumber farming). The workshop was officially opened by the village head of Bweleo. Two members from the Ministry of Agriculture, Livestock and Fisheries were also present. The facilitator addressed opportunities for improving the packaging and marketing of their products in order to enhance sustained benefits throughout their community. Other issues were discussed included initiating temporary closures where women collect bivalves in various sites. This workshop also aimed to strengthen the collaborations between the various villages.

Training courses and workshops for the coastal women involved in aquaculture to build their entrepreneurship skills.

Two big workshop related to this were done during the study period.

The 1st Women's Shellfish Aquaculture Development Workshop was held in Zanzibar on July 29-30th 2015, with 32 women from the villages and other project participants attending. The workshop was facilitated by WIOMSA and IMS through Dr. Narriman Jiddawi and Dr. Maria Haws (co-PI, UHH), also attending the workshop was Dr. Hilary Egan (AquaFish Director), Jenna Borberg (AquaFish Assistant), and Dr. Shivaun Leonard (USAID Washington). Representatives from Sokoine University of Agriculture were also present. The workshop was officially opened by the Ag Director of IMS Dr. M. Mtolera. The workshop provided a venue for exchange of information between IMS and Sokoine personnel, as well as the discussion of many aspects of shellfish culture and alternative livelihoods. Six undergraduate students, who were doing their field work at IMS also attended the training, including one PhD student who is doing a study on mariculture. The workshop was very successful.

Group photo of participants

The 2nd Women's Shellfish Aquaculture Development Workshop was held in Zanzibar on September 29th and 30th, 2017. This workshop brought together participants from coastal villages and technical assistance providers, including one Fisheries Department representative, fish beach recorders, and a representative from a local NGO. Training was provided on bivalve shellfish culture methods. The results of experiments on novel spat collectors from the two villages, Bweleo and Nyamanzi were shared. Participants had the opportunity to offer suggestions for further development of the small-scale bivalve industry. The majority of the participants in the workshop were women (26) since they are the primary participants in shellfish farming and 9 men were also in attendance. In addition to the training on culture methods, training on building entrepreneurship skills and identifying market opportunities were also done. The workshop was officially opened by the Deputy Village Head Bi Mwasiti M. The workshop provided a venue for exchange of information between the villages and their neighbors. The workshop was very successful.

Some participants in the workshop

Participation in exhibitions and other workshops

Through this investigation, the women were given opportunities and supported to attend other workshops relevant to what they are doing as follows:

a. The Ministry of Labour, Empowerment, Elderly, Youth, Women and Children in Collaboration with Ministry of Trade Industry and Marketing, Tanzania Commission for Science and Technology and Voluntary Service Overseas held a two days entrepreneurship workshop on 3rd and 4th of December 2016 in Zanzibar. More than 150m participants were present. The workshop was officially opened by the President of Zanzibar Dr. Ali Mohamed Shein, who encouraged young people to get engaged in entrepreneurship instead of being idle, waiting for government vacancies which can never be sufficient. Researchers and academicians also made presentations on case studies on how to become an entrepreneur and how to organize and manage a business venture in a competitive global marketplace that is constantly evolving. The women we are working with participated in the workshop and learned new things on issues on empowerment along the coast through entrepreneurship. There was also an exhibition where they had the opportunity to present some of their products

b. The women also participated in a one day training workshop on spat collection 20/11/2014. This was done during the visit of Dr. Simon Elis, who visited Zanzibar in November 2014. Ten farmers involved in this project from Nyamanzi and Bweleo villages, attended the training, which was focused on how to make spat collectors and use them in an efficient manner. Swahili guidelines were printed and given to each participant.

Most women in the community don’t know how to swim, so the cooperation of men was important, especially in the diving activities in search of pearl oyster and establishment of the farming long line. The women activities are linked towards seeding the pearls, monthly cleaning the bags used in keeping the oysters submerged in water and jewelry making.

Training on using spat collectors

Long term collection of adult pearl oysters from the wild for pearl production is not environmentally sustainable. However, sustainable pearl production is possible through collection of wild pearl oyster spat using spat collectors. This training also was undertaken to assess settlement of natural occurring oyster spat in the bay. The collectors were hung from the line to 1m depth in the sea. Supervision and monitoring of oyster spat collection cleaning the collectors was left to the community and extension personnel from IMS. This was done twice a month. The exercise was done also as means of not only inspecting but cleaning the collectors. Records were also done on abundance and type of oyster attached in each type of materials used by the community.

During farm visits, it was stressed to the farmers why spat collection was being promoted over continued wild collection of the pearl oysters. There is concern that as the industry grows there will be a growing demand for shells that will put increasing pressure of the wild stocks with the risk of overfishing of pearl shell in the Menai Bay. Spat collection is a more environmentally sustainable way to overcome this problem and also to provide significantly larger numbers of animals for pearl production and jewelry making than wild collection can provide. Additionally, animals grown and held on farms generally produce better quality pearls and shell due a healthier growing environment than in the wild.

Partcipation in international workshops

The facilitators also got opportunities to attend several workshops so as to gain experinces on what others are doing. Dr. Narriman Jiddawi partcipated in the following workshops:

Trip to Ghana July 5 -8, 2013

Dr. Narriman Jiddawi attended the AquaFish Innovation Lab - Africa Regional Meeting at KNUST Engineering Guest House, Kumasi, Ghana in July 2013. This was a collaborative Research Support Program where several key institutions such as Sokoine University of Agriculture, Tanzania; Ministry of Fisheries, Kenya; KNUST, Ghana; Oregon State University, University of Arizona, USA Purdue University, USA; Institute of Marine Sciences Tanzania; Makerere University, Uganda were present. During the workshop, several presentations were made including field visits to Aquaculture cage farms in the Eastern Region and Aquaculture. Some group discussions in developing synergies with other groups working in Africa and description of linkages with other organizations and groups capacity building, technology development, impact assessment, outreach and other elements within each group were made. Include alignment exercises with Mission, USAID, and other groups. The workshop was a great success.

Trip to Indonesia: **Apr 22 – May 1, 2016**

As a host country co-Principal Investigator Dr. N. Jiddawi participated in both the AquaFish Innovation Lab meeting on April 24 – 26, 2016 and the Asia Pacific Aquaculture 2016 Conference, April 27 – 29, 2016. Dr. Maria Haws also was present. The aim of the workshop was to assess project developments and explore possibilities for synergies to develop with other regional AquaFish Innovation Lab projects. During the workshop she gave an oral presentation on sustainable pearl farming in Zanzibar using new spat collection techniques. The presentation discussed various techniques used and the progress made .She also had an opportunity to visit various exhibition and made new connections with new people. The workshop gave a new insight on aquaculture developments in Asia and Africa.

Trip to Cape town South Africa 26th June to 1st July 2017.

As a host country co-Principal Investigator of the project Dr. Narriman S. Jiddawi Senior Lecture at IMS, UDSM participated in both the AquaFish Innovation Lab meeting on 26th June to 1st July 2017 and the Annual International Conference of the World Aquaculture known as the Sustainable Aquaculture New Frontiers for Economic Growth Spotlight on Africa which was held from 25th to 30th June 2017 in Cape Town International Convention Centre, South Africa. The conference was hosted by Aquaculture Association of Southern Africa Department of Agriculture, Forestry and Fisheries, Republic of South Africa. The aim of the workshop was to assess project developments and where each partner has reached. During the workshop she gave an oral presentation on sustainable pearl farming in Zanzibar using new spat collection techniques. All presenters were given certificates of participation. The presentation discussed various techniques used in collection of spats and the progress made so far. The presentation was uploaded in the AquaFish IL web site too. Also she had an opportunity to visit various exhibitions and made new connections with new people. The workshop was sponsored by AquaFish, USA through University of Hawaii, WIOMSA and Sokoine University.

Dr. Jiddawi giving her presentation

Aquaculture America 2018 Las Vegas, Nevada, USA, 71th to 26th Feb 2018

N.S Jiddawi participated in the world Aquaculture America workshop which took place in Las Vegas Nevada between 17th to 26th Feb 2018. As a participant in the workshop, it helped her to learn about matters related to fish aquaculture n and other marine organisms. Also she got an opportunity to get new links and learn from them. She made a presentation on new techniques of spat collectors used in Zanzibar. More than one thousand five hundred people from different countries attended the conference. The sponsors of the workshop were AquaFish Innovation Lab.

Monthly visit to the villages

One trip per month has been done to each of the village involved in pearl farming activity. These are Nyamanzi and Bweleo. The aim was to monitor the pilot spat collectors which have been put on how they were functioning. The spat seem to be attaching quite well. Although it was observed to be much more efficient in Nyamanzi than Bweleo village. This activity was done by the facilitator and an assistant. The aim also was to check if those involved were do the cleaning of the antifouling organisms properly. Also during one of the visits the participants of the workshop which was done in Zanzibar on shell fish a trip was made to the sites to see the area and some of the stakeholders involved.

Once the material was given to the community trips in the villages were undertaken to check on how they were proceeding in making the different types of spat collectors and to see if they were hanging them in the right way. Also a one day field trip was done in the field sites by the AquaFish project participants who were attending the regional meeting which was held at the Sokoine University, Tanzania. The group made a one day visit to Zanzibar on 11th Aug 2014.

The AquaFish group at Fumba Cooperative society building when they visited Zanzibar

Some community members during one of the village visits.

The intertidal areas adjacent to the village are considered heavily overfished with very low harvest rates, therefore the need to start spat collections was found important. Also due to the increasing community demand and need of maintaining sustainable resources utilisation for the benefit of the present and future generation, small scale half pearl farming mariculture development was recommended. This was considered as a means to address poverty and improve the quality of life for coastal communities in Fumba through increasing employment, household incomes, and food security. Trainings were done on oyster spat collection. The training also was undertaken to assess settlement of natural occurring oyster spat in the bay.



Some community participation activities

CONCLUSIONS

Spat collection has previously been tried at the communities of Bweleo and Nyamanzi, with mixed results, using imported spat bags and locally found rubber tires and coconut shells as collectors. Although training was given on how to make and deploy the new black fibrous spat collectors. Farmers were still advised to continue with the coconut shell and rubber tire collectors as these were easily available.

During the training of trainers workshop, participants learned about group formation, how to choose a leader, how to write a constitution for the group and how to write and prepare a business contract. Improving marketing skills has been one of the major focus as it is a big problem.

The workshops highlighted the importance of empowering the women economically. Group discussions were also done to address issues related to the sustainability of spat collection, half pearl farming, and jewelry making.

Several recommendations were discussed by the participants**:**

- Need to have sustainable local and international markets for the half pearl and jewelry produced by the community groups.
- Need to continue with the oyster spat techniques to increase production.

Some problems were also mentioned which if solved could upgrade the pearl farming and jewelry making initiatives. Which were to have a local market and to have a common poit of getting some of their products

ACKNOWLEDGMENTS

We extend our sincere thanks to all who assisted in facilitating this project. Special thanks go to Lali and Ali of Fumba peninsula for working tirelessly in training women to do the spat collators. Our gratitude also goes to the beach recorders, fishers and leaders of the villages of all the two sites for their help during implementation of the work: without them this work would not have succeeded.

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DEVELOPMENT OF LOW-COST AQUAPONIC SYSTEMS FOR KENYA- PART I

Production System Design and Best Management Alternatives/Experiment/13BMA05AU

Julius O. Manyala[1], Kevin Fitzsimmons[2], Charles Ngugi[3], Josiah Ani[1], and Elizabeth Obado[1]

[1]*University of Eldoret, Kenya*
[2]*University of Arizona, USA*
[3]*Kenyatta University, Nairobi, Kenya*

ABSTRACT

The activities of the University of Eldoret involved the designing and testing of small-scale low-cost aquaponics system that can be used for training and extension. This system is specifically suitable for small-scale fish hobbyists in water deficient situations and urban/semi urban areas where land is scarce. The small-scale was developed and its efficiency assessed using different fish stocking densities. The small-scale aquaponic system consisted of a rectangular fish culture tank rising to 460 mm from the bottom and a plant bed rising to 270 mm from a raised platform, both units being arranged in a vertical tier. Water overflow from the fish unit was passed through a bio-filter made of shredded plastic material to increase the surface area. These units acted as a nitrification chamber before the water was flowed by gravity into the plant beds. The effluent water from the plant beds was pumped back to the fish tank units using a submersible lift pump for the small-scale system as shown in the appendices. Water discharge from the plant unit flows back to the fish unit by gravity thereby elimination the need for double pumping in the small-scale system. The prototype units were constructed at University of Eldoret and were tested using all male tilapia (*Oreochromis niloticus*) fry for 42-105 days to the fingerling and juvenile stages. Results from the trial show that fish stocking density has an effect on the nutrient budget of the system. High nitrate content in the fish unit was associated with high stocking density of 80 fish per tank as compared to 60 Fish per tank for the small-scale system and 150 kg as compared to 100 kg in the medium-scale system. The nitrification unit exhibited high efficiency since ammonia was not detected in the plant beds in both systems. Quantities of ammonia detected in the fish tanks after 35 days was close to the target values of zero. All-important water quality parameters for both the aquaponic systems such as DO, pH, alkalinity and Temperature were within optimum values. The results facilitated the development of a moderate-scale aquaponic system that was tested in the field in collaboration with local farmers though they preferred to use the African catfish and kales due to personal preferences. It is concluded that the systems is viable and self-regulating in terms of nitrogen cycle. The only limiting factor is the provision of other nutrients required for plant growth by supplemental fertilization. Farmers have opted to overcome nutrient limitations in the plant beds by adopting gravel plant bed units instead of floating rafts. This report provides both design specifications and technical drawings of the aquaponics system developed during this activity. The unit offers good opportunities for rapid commercialization by the private entrepreneurs but there is need to improve on energy requirement through solar technology.

INTRODUCTION

Millions of people around the world find a source of income and livelihood in the fisheries and aquaculture sector. Recent estimates indicate that 58.3 million people were engaged in the primary sector of capture fisheries and aquaculture in 2012 (Somerville et al. 2014). Fisheries and aquaculture play important roles in providing food and income in many developing countries, either as a stand-alone activity or in association with crop agriculture and livestock rearing. The harvest, sale and processing of fish contribute indirectly to food security by increasing purchasing power at individual or household level, nationally and also regionally. Demand for fish as source of protein is expected to increase substantially, at least in line with other animal-based food products, particularly in South and South-East Asia (Allison et al. 2015).

Current global per-capita supply of fish is 17 kg per year; nearly half of this supply comes from aquaculture (Somerville et al. 2014). The availability of fish is unevenly distributed, with supply constraints faced by some undernourished populations in developing countries with high dependence on fish, particularly in sub-Saharan Africa, the least developed countries of South and South East Asia, and small island states in the Pacific Ocean (Allison et al. 2015; Frediani 2011).

There is growing need for innovative production methods to enhance production of fish from the wild and through technology-enhanced aquaculture. Aquaponics, the integrated culture of fish and other aquatic organisms with plants is one such technology which has gained considerably mileage in areas with water scarcity. However, this technology remains largely un-tapped in Kenya and much of Africa.

The potential of an opportunity for Aquaponics are several and includes: its contribution to community transformation, Aquaponics industry development, industrial change and development and the implementation of policies and programs on food security, technology and income generation within many economic contexts in Sub-Sahara Africa. Though it has a great potential for Kenya and other developing countries, Aquaponics is a young science and the development of newer technology in the field is still progressing (IBM Report 2011).

Aquaponics describes the combination of two principal growing processes working in harmony to deliver one, self-sustained and ecologically balanced culture system; aquaculture and hydroponics. Aquaculture component involves farming of aquatic animals, in controlled marine or fresh water environments. The hydroponics component involves growing edible plants within the unit. The idea is to combine these techniques together within the same system, so that the positives of both are multiplied and negatives of each are minimized by each unit.

The integrated system of aquaponics has benefits not achievable when aquaculture and hydroponics are applied separately (Timmons and Ebeling 2010). In fact existing production units have demonstrated that aquaponics permits the producer to be more efficient with water, energy, and to protect the crops from soil borne diseases. Furthermore, aquaponics can bring a new approach to the sustainability of landscapes, urban agriculture and the sustainability of cities by turning wastes into resources and transforming disused urban spaces to provide not only food, but resilient resilience to many possible livelihood shocks (Price 2009).

Theoretically, the nutrient content of a diet used in aquaponics can be manipulated to make the relative proportions of nutrients excreted by fish more similar to the relative proportions of nutrients assimilated by the plant component. There must then be an optimal fish to plant ratio, however, this ratio depends on the plant and fish and often requires experimentation to determine (Price 2009; Singh *et al.* 1999). This project therefore aimed at investigating the performance of Nile tilapia (*Oreochromis niloticus*) under an aquaponics system as a means to increase productivity and control the usually harmful effects of waste water from the traditional aquaculture systems.

OBJECTIVES

1. Design a small-scale aquaponic system for educational purposes and hobby production of fish and vegetables.
2. Construct a small-scale system to develop proof of concept and training.

MATERIALS AND METHODS

The study was conducted at the University of Eldoret at the hatchery unit. The experimental protocol involved the growth trials of all monosex *Oreochromis niloticus* fry for 5 weeks to attain the fingerlings stage with lettuce in the hydroponics system. All the fry were obtained from Sagana National Aquaculture Research and Development Centre (NARDC). The second study was conducted in a greenhouse with the medium-scale aquaponic system.

Design of small-scale and medium-scale aquaponic systems

Preparation of floating Styrofoam

Styrofoam of dimensions 1m x 0.5m x 0.03m (length, width and thickness) were used. Each Styrofoam sheet had 8 evenly drilled holes of diameter 4 cm. The sheets were placed on in the hydroponic system. This is where the plants were anchored. Each of the drilled holes had a plastic plant pot which was used to support and suspend the plants. The pots had 6-9 open strips to allow plant roots to freely develop and tap the nutrients.

Source of plants

Lettuce seeds used in this experiment were sourced from a reputable agro vet shop in Eldoret. The seeds were then planted in plastic trays placed in a greenhouse. The seeds were carefully inserted in wet cotton sheets to allow them germinate. After 8 days all the seeds germinated. The germinated plants were immediately introduced into nursery hydroponic system units where they grew and developed roots fully for a period of 7 more days. All the healthy plants with well-developed roots were uprooted and planted in the in the rafts.

Experimental design

The study was done using completely randomized design (CRD). Nine tanks of 100 L were used during this experiment. Two aquaponic treatments were each stocked with Monosex Nile tilapia fry at stocking densities of 60 fry/tank and 80 fry/tank, respectively, and replicated four times. Each treatment was subjected to lettuce plants from the University of Eldoret Horticulture Department at a density of plants 16 per m2.

Experimental setup

The experiment to test the aquaponics system was set up using a randomized block design as outlined in Table 1. The hydroponic unit consisting of the plant beds was independently attached to each of the fish tanks.

Table 1. Experimental setup: treatments

Tank and Stocking Density		Tank and Stocking Density		Tank and Stocking Density	
Tank 1 =	80 Fish	Tank 2 =	60 Fish	Tank 3 =	80 Fish
Tank 4 =	60 Fish	Tank 5 =	80 Fish	Tank 6 =	60 Fish
Tank 7 =	80 Fish	Tank 8 =	80 Fish	Tank 9 =	60 Fish

Feed preparation

The fingerlings were fed with locally formulated feeds of crude protein 30%. The constituents of the feed used included wheat bran, rice polish, *Rastrineobola argentea* fish meal, and cottonseed cake. The sun-dried *R. argentea* were bought from Kisumu market. All ingredients was ground individually into fine powder using an electrical grinding mill, measured in the respective proportions then mixed and subjected to proximate analysis. The proximate analysis was determined at the University of Eldoret Fisheries laboratory following procedures described by AOAC (1984). Equal proportions of sunflower oil and cod liver oil (1:1) were added as lipid source in the test diets.

Data collection: *Water quality*

Data for dissolved oxygen, pH and temperature were collected daily in the recirculating fish rearing tank, hydroponic tank and lastly the sump. Once a week the nutrient load in the three main sections of the system was also be monitored and recorded. Parameters checked were ammonia, nitrates, dissolved oxygen pH, and temperature. This was done using water test kits, YSI DO and temperature Meter (Y540) and pH meter Y333.

Nitrates and Ammonia were analyzed using the following procedures respectively:

i) An EPA and ASTM approved and preferred method for estimating nitrate in water is the Strickland and Parsons (1968) Cadmium Column Reduction Method.
ii) Ammonia by direct nesslerization (APHA, AWWA, WEF 2012).

Fish growth

Random samples of 30% fish stocked in each tank were taken from each of the nine tanks for weight and length measurement after every week. On the first sampling occasion the fry were weighed together on an electronic balance (readability 0.01 mg, model VI-200) and average weight computed. The lengths of each fry were then measured using a meter rule to the nearest 0.1 mm.

Plant data collection

Plant height: After every three days the height of individual plants was measured in centimeters. The height was from the Styrofoam surface to the top of the main plant stem. Leaf numbers -- the number leaves of leaves for each plant were counted. The tips of newly emerging plants was also counted and recorded.

Leaf length: The lengths of individual leaves were measured in centimeters. The length was from the base of the leaf to the tip of the leaves. The width of individual leaves was also taken and recorded.

Data analysis

The general water quality parameters -- dissolved oxygen, pH, temperature and alkalinity -- were subjected to 2-sample t-test among treatments. Treatments were the different fish stocking densities per tank (80 Fish/T and 60 Fish/T).

The amount of Nitrates in the fish rearing unit and in the plant rearing units over time were compared using regression slopes with treatments as factors. Ammonia concentrations were also compared over time, using regression among treatments as factors for the fish tank only since it was not detectable in the plant rearing units.

Fish growth in length and plant growth in height against time were compared using regression slopes among treatments on assumption of linear relationship for fry-fingerling growth phase and plant height. The slopes provided an indication of growth rate between the 80 Fish/T and 60 Fish/T treatments for fish as well as for plants respectively.

Lastly, a Neural Network Bayesian classifier was used to verify the robustness of the results by classifying all the observations according to treatment and using Nitrates, Ammonia and Alkalinity as factors in the model.

RESULTS

General water quality parameters for aquaponics

The critical water parameters in the aquaponics system; Dissolved Oxygen, Temperature, pH and Alkalinity were not significantly different on a weekly basis nor were they significantly different throughout the experiment (Table 2).

Table 2. Mean±SE of general water quality parameters required for balancing aquaponics system taken over the experimental period.

Time (Weeks)	60 Fish	80 Fish	2-Sample t-test
		Dissolved Oxygen	
Week 1	4.24±0.488	5.10±0.442	$t_{0.05,8}$ = 0.406499; p-value = 0.69
Week 2	5.25±0.266	5.45±1.001	$t_{0.05,8}$ = -0.147370; p-value = 0.88
Week 3	5.09±0.386	5.17±0.871	$t_{0.05,8}$ = -0.136778; p-value = 0.89
Week 4	5.47±0.464	5.33±0.776	$t_{0.05,8}$ = 0.406499; p-value = 0.69
Week 5	4.97±0.283	5.37±0.888	$t_{0.05,8}$ = 0.034579; p-value = 0.97
		Temperature	
Week 1	17.73±0.131	17.66±0.254	$t_{0.05,8}$ = 0.349459; p-value = 0.73
Week 2	18.45±0.240	18.28±0.260	$t_{0.05,8}$ = 0.143007; p-value = 0.89
Week 3	18.18±0.197	17.70±0.184	$t_{0.05,8}$ = -0.445821; p-value = 0.66
Week 4	17.85±0.240	17.78±0.237	$t_{0.05,8}$ = 0.349459; p-value = 0.73
Week 5	18.23±0.149	17.68±0.193	$t_{0.05,8}$ = -0.391346; p-value = 0.70
		pH	
Week 1	8.01±0.164	8.11±0.159	$t_{0.05,8}$ = -0.413396; p-value = 0.69
Week 2	8.15±0.169	8.51±0.231	$t_{0.05,8}$ = -1.211390; p-value = 0.26
Week 3	8.16±0.167	8.47±0.221	$t_{0.05,8}$ = -1.057100; p-value = 0.32
Week 4	8.06±0.111	8.16±0.194	$t_{0.05,8}$ = -0.426447; p-value = 0.68
Week 5	8.18±0.177	8.44±0.249	$t_{0.05,8}$ = -0.828957; p-value = 0.43
		Alkalinity	
Week 1	183.83±4.275	201.90±6.415	$t_{0.05,8}$ = -2.208160; p-value = 0.06
Week 2	192.25±8.138	201.64±6.366	$t_{0.05,8}$ = -0.924325; p-value = 0.38
Week 3	175.23±10.712	170.96±9.348	$t_{0.05,8}$ = 0.300927; p-value = 0.77
Week 4	175.33±10.765	177.76±8.696	$t_{0.05,8}$ = -0.178245; p-value = 0.86
Week 5	175.28±10.679	194.84±8.680	$t_{0.05,8}$ = -1.439040; p-value = 0.19
		Individual Parameters	
Dissolved Oxygen	5.66±0.598	5.5472±0.603	$t_{0.5,43}$ = 0.2715090; p-value = 0.78
Temperature	20.94±0.280	20.928±0.325	$t_{0.5,43}$ = 0.0797688; p-value = 0.93
pH	8.11± 0.136	8.3384±0.193	$t_{0.5,43}$ = -1.897030; p-value = 0.06
Alkalinity	180.38±8.368	189.42±8.615	$t_{0.5,43}$ = -1.536880; p-value = 0.13

Nitrate in fish tanks

The output shows the results of fitting a linear regression model to describe the relationship between Nitrate, Days and Treatment in the fish tanks. The equation of the fitted model is:

$$Nitrate = 75.3 - 1.09 \times Days + 21.71 \times (Treat = 80\ Fish) - 0.62 \times Days \times (Treat = 80\ Fish)$$

Where the terms similar to Treatment=80 Fish are indicator variables which take the value 1 if true and 0 if false. This corresponds to 2 separate lines, one for each value of Treatment. For example, when Treatment=60 Fish, the model reduces to:

$$Nitrate = 75.3 - 1.09 \times Days$$

When treatment=80 fish, the model reduces to:

$$Nitrate = 97.0 - 1.71 \times Days$$

Because the p-value in the ANOVA table (F0.5,3,41=344.3; p-value<0.00005), was less than 0.05, there was a statistically significant relationship between the variables at the 95.0% confidence level

(Table 3). The R-Squared statistic indicates that the model as fitted explains 96.2% of the variability in Nitrate.

The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of independent variables, was 95.9%. The mean absolute error (MAE) was 2.5 and is the average value of the residuals. The Durbin-Watson (DW) statistic used tests the residuals to determine any significant correlation based on the order in which they occur in the data file was 1.4 (p-value=0.0045) showing that there is no indication of possible serial correlation at the 95.0% confidence level.

Table 3. ANOVA for regression of nitrates on time by treatment in fish unit.

Source	Sum of Squares	df	Mean Square	F-Ratio	p-value
Model	10331.1	3	3443.69	344.27	0.00005
Residual	410.115	41	10.0028		
Total (Corr.)	10741.2	44			
R-Squared = 96.18 percent			R-Squared (adjusted for d.f.) = 95.9 percent		
Standard Error of Est. = 3.162			Mean absolute error = 2.448		
Durbin-Watson statistic = 1.391 (P=0.0048)			Lag 1 residual autocorrelation = 0.299		

Statistical test (F0.5,1,3=344.3; p-value<0.00005) show that the slopes for treatment were statistically significant at 99% confidence level because the p-value for the slopes is less than 0.01. Because the p-value for the intercepts is less than 0.01, there are statistically significant differences among the intercepts for the various values of Treatment at the 99% confidence level (Table 4 and Figure 1).

Table 4. ANOVA for variables in the order fitted for regression of nitrates on time by treatment in the fish unit.

Source	Sum of Squares	df	Mean Square	F-Ratio	p-value
Days	9080.18	1	9080.18	907.76	0.00005
Intercepts	829.44	1	829.44	82.92	0.00005
Slopes	421.467	1	421.467	42.13	0.00005
Model	10331.1	3			

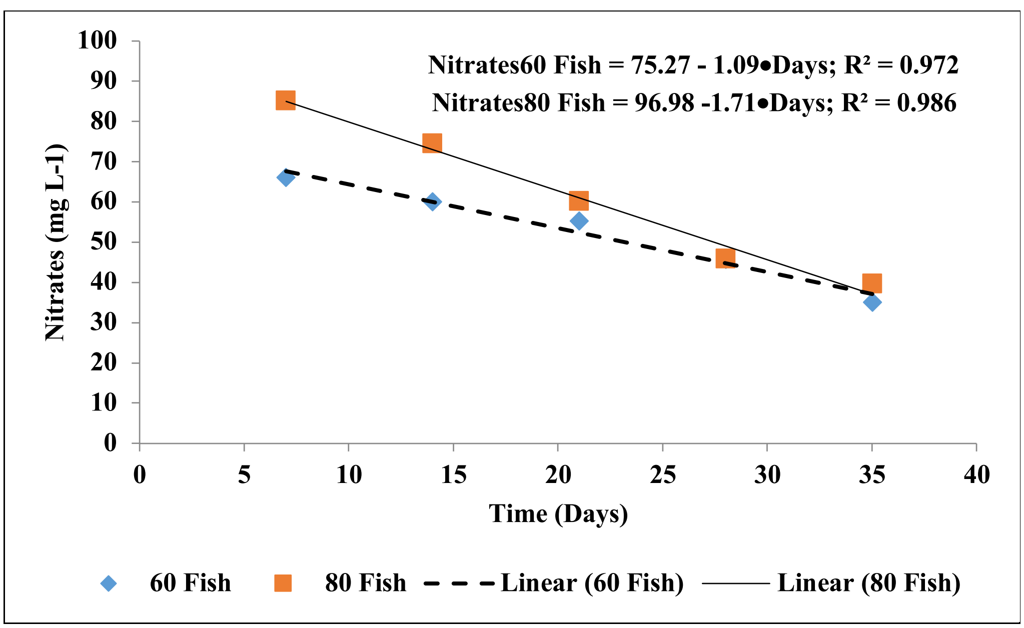

Figure 1. Nitrate concentration in the fish rearing tanks during the experimental period showing a decline for both treatments.

Nitrate in Hydroponic Units

The output shows the results of fitting a linear regression model to describe the relationship between Nitrate, Days and Treatment in the hydroponics tanks. The equation of the fitted model is:

$$Nitrate = 0.20475 - 0.00068 \times Days + 0.34125 \times (Treat = 80\ Fish) - 0.004521 \times Days \times (Treat = 80\ Fish)$$

Where the terms similar to Treatment=80 Fish are indicator variables which take the value 1 if true and 0 if false. This corresponds to 2 separate lines, one for each value of Treatment. For example, when Treatment=60 Fish, the model reduces to:

$$Nitrate = 0.20475 - 0.00068 \times Days$$

When Treatment=80 Fish, the model reduces to:

$$Nitrate = 0.546 - 0.0052 \times Days$$

Because the p-value in the ANOVA table (F0.5,3,41=62.7; p-value<0.00005), was less than 0.05, there was a statistically significant relationship between the variables at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains 82.1% of the variability in P-Nitrate. The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of independent variables, was 80.8%. The mean absolute error (MAE) was 0.063 and is the average value of the residuals. The Durbin-Watson (DW) statistic used tests the residuals to determine any significant correlation based on the order in which they occur in your data file was 1.93 (p-value=0.24) show that there is an indication of possible serial correlation at the 95.0% confidence level (Table 5).

Table 5. ANOVA in regression of nitrates on time by treatment in plant unit.

Source	Sum of Squares	df	Mean Square	F-Ratio	p-value
Model	0.74	3	0.2471	62.65	0.00005
Residual	0.16	41	0.0039		
Total (Corr.)	0.90	44			
R-Squared = 82.09 percent			R-Squared (adjusted for d.f.) = 80.78 percent		
Standard Error of Est. = 0.062			Mean absolute error = 0.0498378		
Durbin-Watson statistic = 1.933 (p-value=0.24)			Lag 1 residual autocorrelation = 0.029		

Statistical test ($F_{0.5,1,3}$=5.64; p-value<0.02) show that the slopes for treatment were statistically significant at 95% confidence level because the p-value for the slopes is less than 0.05 (Table 6).

Table 6. ANOVA for variables in the order fitted for regression of nitrates on time by treatment in the plant unit.

Source	Sum of Squares	df	Mean Square	F-Ratio	p-value
Days	0.04489	1	0.04489	11.38	0.0016
Intercepts	0.67404	1	0.67404	170.92	0.00005
Slopes	0.02226	1	0.02226	5.64	0.0223
Model	0.74119	3			

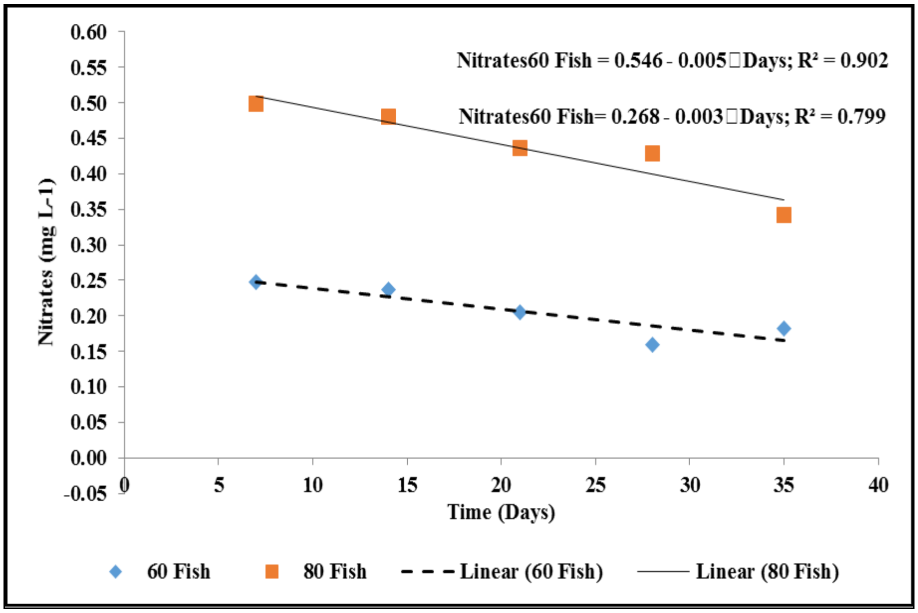

Figure 2. Nitrate concentration in the plant rearing tanks during the experimental period showing a decline for both treatments.

Ammonia in fish tanks

The output shows the results of fitting a linear regression model to describe the relationship between Ammonia, Days and Treatment in the fish tanks. The equation of the fitted model is:

$$Amm = 0.0027 - 0.0000087 \times Days + 0.0175 \times (Treat = 80\ Fish) - 0.00061 \times Days \times (Treat = 80\ Fish)$$

Where the terms similar to treatment=80 fish are indicator variables which take the value 1 if true and 0 if false. This corresponds to 2 separate lines, one for each value of Treatment. For example, when Treatment=60 Fish, the model reduces to:

$$Amm = 0.0027 - 0.000087 \times Days$$

When Treatment=80 Fish, the model reduces to

$$Amm = 0.0202 - 0.0007 \times Days$$

Because the p-value in the ANOVA table ($F_{0.5,3,41}=11.6$; p-value<0.00005), was less than 0.05, there was a statistically significant relationship between the variables at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains only 45.91% of the variability in ammonia. The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of independent variables, was 41.95%.

The MAE was 0.0065 and is the average value of the residuals. The DW statistic used tests the residuals to determine any significant correlation based on the order in which they occur in your data file was 0.764 (p-value<0.00005) show that there is an indication of possible serial correlation at the 95.0% confidence level (Table 7).

Table 7. ANOVA for regression of ammonia on time by treatment in fish unit.

Source	Sum of Squares	df	Mean Square	F-Ratio	p-value
Model	0.00145539	3	0.000485129	11.60	<0.00005
Residual	0.00171476	41	0.0000418234		
Total (Corr.)	0.00317014	44			

R-Squared = 45.90 percent
Standard Error of Est. = 0.006
Durbin-Watson statistic = 0.763 (p-value<0.00005)
R-Squared (adjusted for d.f.) = 41.95 percent
Mean absolute error = 0.003
Lag 1 residual autocorrelation = 0.612

Statistical test (F0.5,1,3=344.3; p-value=0.003) show that the slopes for treatment were statistically significant at 99% confidence level because the p-value for the slopes is less than 0.01. Because the p-value for the intercepts is less than 0.05, there are statistically significant differences among the intercepts for the various values of Treatment at the 95% confidence level (Table 8 and Figure 3).

Table 8. ANOVA for variables in order fitted for regression of ammonia on time by treatment in the fish unit.

Source	Sum of Squares	df	Mean Square	F-Ratio	P-Value
Days	0.000807404	1	0.000807404	19.31	0.0001
Intercepts	0.000237653	1	0.000237653	5.68	0.0218
Slopes	0.00041033	1	0.00041033	9.81	0.0032
Model	0.00145539	3			

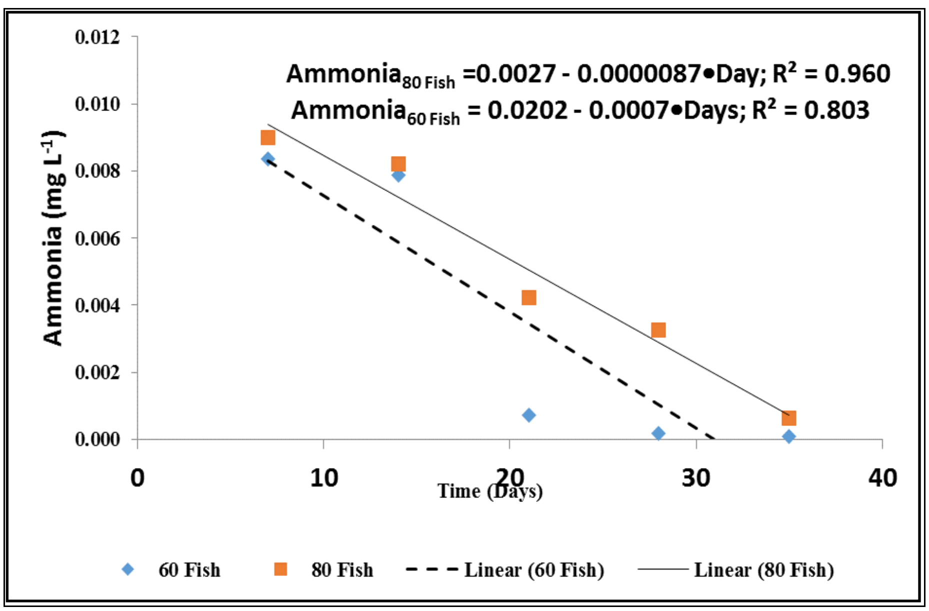

Figure 3. Nitrate concentration in the plant rearing tanks during the experimental period showing a decline for both treatments.

Fish growth in length

The output shows the results of fitting a linear regression model to describe the relationship between Fish Length, Days and Treatment in the fish tanks. The fitted model equation is:

$$Length = 3.24 + 0.83 \times Days + 2.1 \times (Treat = 80\ Fish) - 0.23 \times Days \times (Treat = 80\ Fish)$$

Where the terms similar to Treatment=80 Fish are indicator variables which take the value 1 if true and 0 if false. This corresponds to 2 separate lines, one for each value of Treatment. For example, when Treatment=60 Fish, the model reduces to:

$$Length = 3.24 + 0.83 \times Days$$

When Treatment=80 Fish, the model reduces to:

$$Length = 5.33 + 0.60 \times Days$$

Because the p-value in the ANOVA table ($F_{0.5,3,41}=399.46$; p-value<0.00005), was less than 0.05, there was a statistically significant relationship between the variables at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains only 96.69% of the variability in ammonia. The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of independent variables, was 96.5%.

The MAE was 1.18 and is the average value of the residuals. The DW statistic used tests the residuals to determine any significant correlation based on the order in which they occur in the data was 0.631 (p-value<0.00005) show that there is an indication of possible serial correlation at the 95.0% confidence level (Table 9).

Table 9. ANOVA for variables in the order fitted for regression

Source	Sum of Squares	df	Mean Square	F-Ratio	P-Value
Model	2326.44	3	775.481	399.46	<0.00005
Residual	79.5947	41	1.94134		
Total (Corr.)	2406.04	44			

R-Squared = 96.69 percent
R-Squared (adjusted for d.f.) = 96.44 percent
Standard Error of Est. = 1.393
Mean absolute error = 1.178
Durbin-Watson statistic = 0.630 (p-value<0.00005)
Lag 1 residual autocorrelation = 0.655

Statistical test ($F_{0.5,1,3}=30.68$; p-value<0.00005) show that the slopes for treatment were statistically significant at 99% confidence level because the p-value for the slopes is less than 0.01. Because the p-value for the intercepts is less than 0.01, there are statistically significant differences among the intercepts for the various values of Treatment at the 99% confidence level (Table 10 and Figure 4).

Table 10. ANOVA for variables in the order fitted.

Source	Sum of Squares	df	Mean Square	F-Ratio	P-Value
Days	2178.77	1	2178.77	1122.31	<0.00005
Intercepts	88.1095	1	88.1095	45.39	<0.00005
Slopes	59.5613	1	59.5613	30.68	<0.00005
Model	2326.44	3			

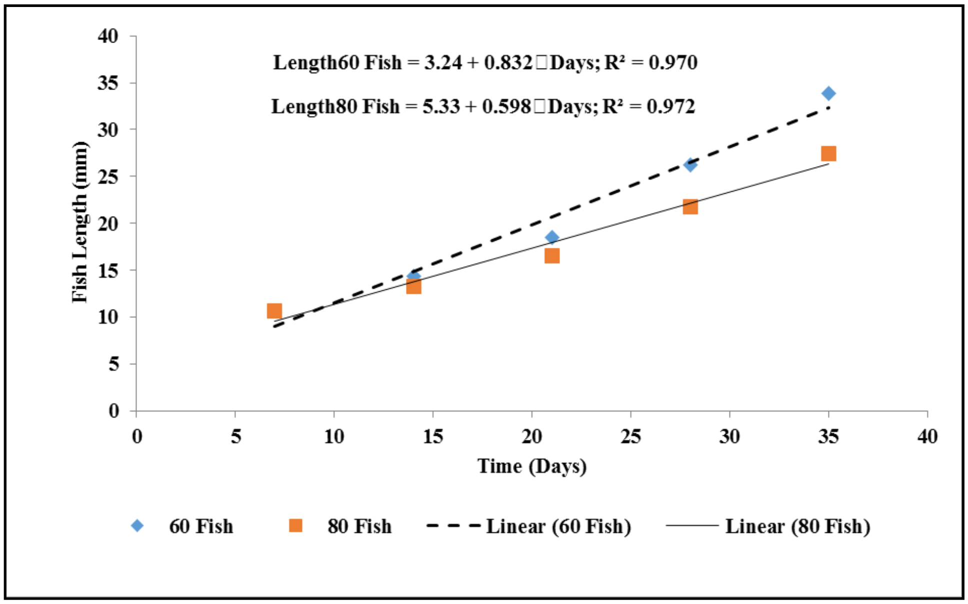

Figure 4. Size variation in length (mm) of monosex *O. niloticus* fry with time and treatment.

Growth in plants

The output shows the results of fitting a linear regression model to describe the relationship between Plant Height, Days and Treatment in the fish tanks. The equation of the fitted model is:

$$Height = 12.12 + 0.97 \times Days - 9.76 \times (Treat = 80\ Fish) + 1.46 \times Days \times (Treat = 80\ Fish)$$

Where the terms similar to Treatment=80 Fish are indicator variables which take the value 1 if true and 0 if false. This corresponds to 2 separate lines, one for each value of Treatment. For example, when Treatment=60 Fish, the model reduces to:

$$Height = 12.12 + 0.97 \times Days$$

When Treatment=80 Fish, the model reduces to:

$$Height = 2.36 + 2.43 \times Days$$

Because the p-value in the ANOVA table (F0.5,3,41=177.77; p-value<0.00005), was less than 0.05, there was a statistically significant relationship between the variables at the 95% confidence level. The R-Squared statistic indicates that the model as fitted explains only 92.9% of the variability in ammonia. The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of independent variables, was 92.3%. The MAE was 3.39 and is the average value of the residuals. The DW statistic used tests the residuals to determine any significant correlation based on the order in which they occur in your data file was 2.62 (p-value=0.955) show that there is no indication of possible serial correlation at the 95.0% confidence level (Table 11).

Table 11. ANOVA for variables in the order fitted for regression

Source	Sum of Squares	df	Mean Square	F-Ratio	P-Value
Model	21215.1	3	7071.69	177.77	<0.00005
Residual	1630.94	41	39.7789		
Total (Corr.)	22846.0	44			

R-Squared = 92.86 percent
R-Squared (adjusted for d.f.) = 92.3 percent
Standard Error of Est. = 6.30705
Mean absolute error = 3.38738
Durbin-Watson statistic = 2.62124 (p-value=0.9551)
Lag 1 residual autocorrelation = -0.315011

Statistical test (F0.5,1,3=58.36; p-value<0.00005) show that the slopes for treatment were statistically significant at 99% confidence level because the p-value for the slopes is less than 0.01. Because the p-value for the intercepts is less than 0.01, there are statistically significant differences among the intercepts for the various values of treatment at the 99% confidence level (Table 12 and Figure 5).

Table 12. ANOVA for variables in the order fitted for regression

Source	Sum of Squares	df	Mean Square	F-Ratio	P-Value
Days	14038.8	1	14038.8	352.92	<0.00005
Intercepts	4854.9	1	4854.9	122.05	<0.00005
Slopes	2321.42	1	2321.42	58.36	<0.00005
Model	21215.1	3			

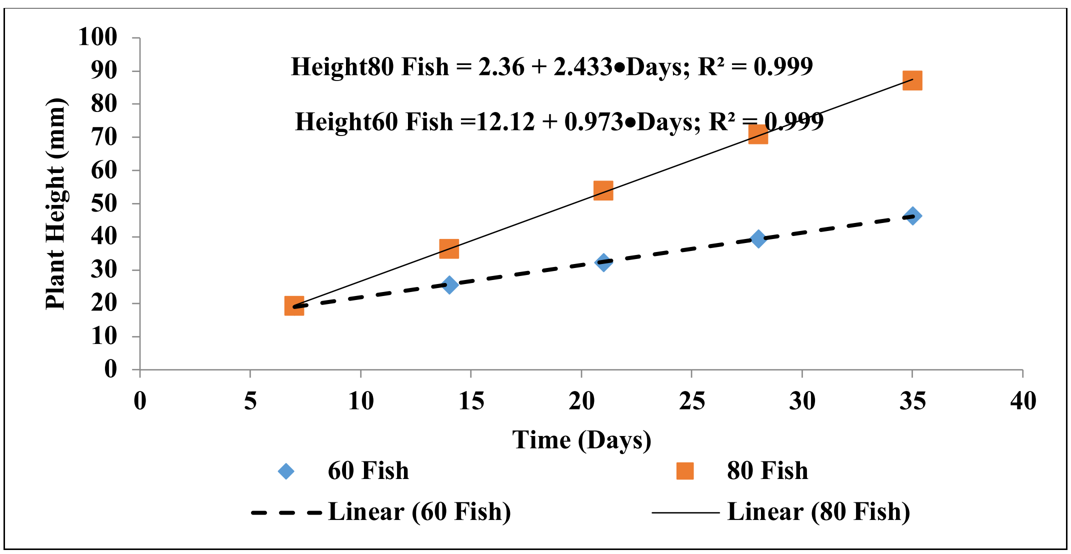

Figure 5. Size variation in height (mm) of lettuce with time and treatment.

Neural network Bayesian classifier for treatment

Four input factors; Nitrates in the fish tanks (F-Nitrate), ammonia in the fish tanks (Ammonia), Nitrates in the hydroponics units (Nitrate), and alkalinity, and using a probabilistic neural network (PNN) classified 100% of the treatment cases correctly. The best architecture for the network was 4-45-2-2 (Figure 6).

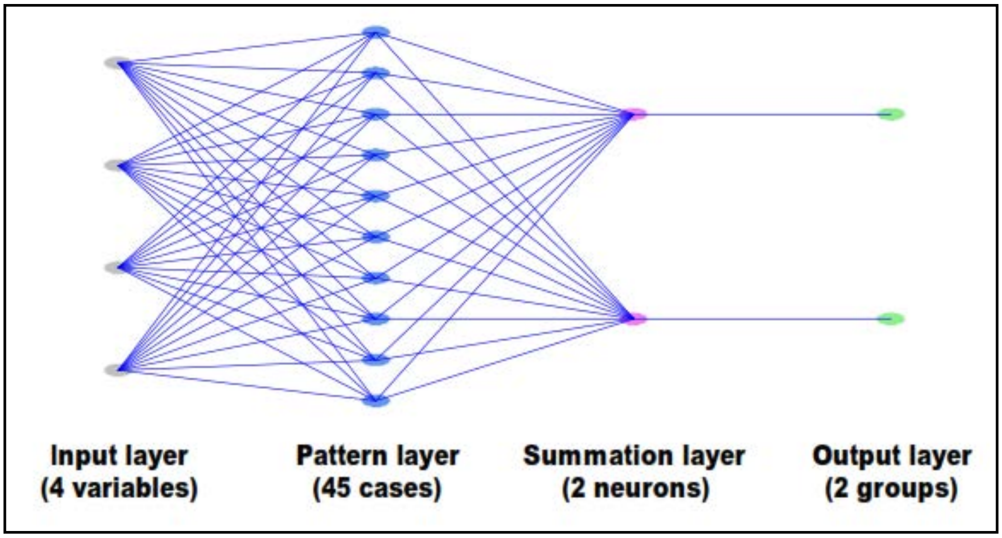

Figure 6. ANN Bayesian classifier 4-45-2-2 architecture for the treatment in the aquaponics system.

A constructed scatter plot of nitrates in the fish tanks and hydroponics units and ammonia in the fish tanks show relatively low values of all these parameters in the 60 Fish/T as compared to 80 Fish/T treatment (Figure 7).

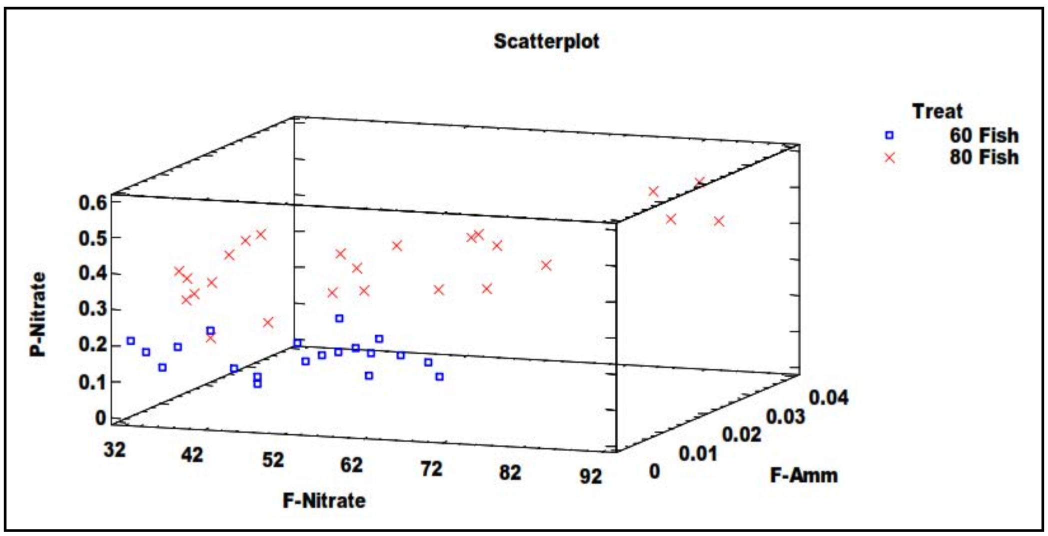

Figure 7. Scatter plot of nitrates and ammonia concentrations in the aquaponics system.

CONCLUSION

The theoretical concept in an aquaponics system is to convert the ammonia (NH3) in fish waste into nitrite (NO2-). Then nitrite (NO2-) is transformed into nitrate (NO3-) to be used in the plant bed (Somerville et al. 2014).

There are two major groups of nitrifying bacteria involved in the nitrification process: i) Ammonia Oxidizing Bacteria (AOB) that converts Ammonia (NO3) to nitrate (NO2-), commonly the genus Nitrosomonas and ii) Nitrite Oxidizing Bacteria (NOB) converts nitrite (NO2-) to nitrate (NO3-), commonly the genus Nitrobacter. Since the aquaponics system is totally reliant on the bacteria and the nitrogen cycle, the present study measures and analyzes these dimensions of nitrogen in both the aquaculture and the hydroponics units.

Main findings Since the values of dissolved oxygen were within the expected (4 - 8 mg L-1), nitrates also were in the expected range (<400 mg L-1), temperatures were 20.93±0.33 to 20.94 ±0.28 °C. The alkalinity target is 60-140 mg L-1, so it can be concluded that the water quality balance was within the conducive range for an aquaponics system.

The nitrate levels is expected to be 5 to 150 mg L-1 and in the current study, values obtained in both the aquaculture and hydroponics were ranging between 30 and 90 mg L-1 and between 0.15 and 0.55 mg L-1 respectively, it can be concluded that the system was optimized with the present levels of stocking, feeding and flow rate. The slightly high levels of nitrate in the aquaculture unit can be attributed to feed inputs. Although both treatments seemed to be efficient, there was initially higher levels of nitrates in the aquaculture unit but the concentration declined at a faster rate in the 80 Fish/T treatment (b=1.71) as compared to 60 Fish/T (b=1.09). This difference can be attributed to the differences in the amount of feed and feed utilization. Similarly in the hydroponics unit, there were slightly higher level of nitrates indicating the impact of external inputs to the fish tanks, assuming equal efficiency in the nitrification unit or biofilter.

Ammonia levels of below 0.01 mg L-1 can be considered as negligible in view of the time required for an aquaponic system to stabilize. In this study, ammonia was undetectable in the hydroponic unit and this observation has led to the belief that the nitrification system performed optimally in both treatments.

Significant differences in fish growth could be attributed to space and stocking density rather than the performance of the aquaponics system. Since 60 Fish/T grew at a faster rate than 80 Fish/T, stocking density has to be adjusted for the present prototype. The overall growth was considered adequate since monosex *O. niloticus* fry were raised to fingerlings of about 4 cm in 4-5 weeks. In practice, tilapia fingerling producers in Kenya take about the same time to raise fingerlings in hapas at relatively elevated temperatures of 24 °C as compared to 20.9 °C in this study.

The high nutrient (nitrate) in the 80 Fish/T (b=2.43) treatment is adequately reflected in significantly better plant growth as compared to 60 Fish/T (b=0.97). The preliminary results hence provide an indication of the potential to produce fish and crops using this system.

The study constructed an Artificial Neural Network Bayesian Classifier (ANN-B Classifier) to validate the impact of nitrates, ammonia and alkalinity as factors associated with the two treatments. This validation approach allows simulation of input factors to predict new observations. Even though the predictive ability of ANN-B Classifier is rare in biological sciences, it offers a more robust data grouping method than the conventional Discriminant Function Analysis (DFA) and Multi-Dimensional Scaling (MDS).

Main understandings-Based on the results in the study, it is concluded that:

i. The prototype aquaponics system developed in this study can be used to raise both fish and crops within the tropical setup and especially in water deficient East Africa.
ii. A single unit is presented in the report to show the basic design for small scale production when 2 to 5 units are connected in series or in parallel. Medium-scale production system involves connecting a series of the units in parallel or in series. The dynamics of the medium-scale system is kept at equilibrium by maintaining the measurements and specification of the single units.
iii. The nitrate nutrient cycle in the system is balanced and optimized for efficiency, assuming some critical water quality parameters are controlled.
iv. Both fish and horticultural crops had satisfactory growth performance in the prototype aquaponics system developed during this study.

v. The biofilter system developed in the study is capable of efficient nitrification to provide required nitrates for the plant bed.

QUANTIFIED ANTICIPATED BENEFITS

This project has been instrumental in the following ways:

i. One Ph.D. student has used the opportunity to collect preliminary data for his thesis hence contributing to knowledge on this un-explored opportunity for aquaculture in water deficient areas and land limiting urban/semi urban areas.
ii. The Aquaponics Project has used feeds formulated and processed by a M.Sc. Student working on fish feeds for juvenile tilapia (*O. niloticus*) and hence complemented the testing of on-farm formulated feeds.
iii. The demonstration unit was in a high visibility location and received attention from faculty, staff, students, and local area farmers. We expect that at least 200 individuals observed the workings of the unit. The fish and vegetables produced were consumed by students or sold to generate funds for student activities.
iv. The unit was for farmers who have ponds and wish to use the nutrient enriched water to irrigate field and vegetable crops. We document the increased levels of nitrogen that can contribute to fertilizing plants and reducing the costs for chemical fertilizers for farmers.

ACKNOWLEDGMENTS

We acknowledge AquaFish Innovation Lab for the initial funding of this project. AquaFish funding was used to leverage additional funding from the National Council for Science, Technology and Innovation (NACOSTI) to acquire further equipment for water quality and nutrient analysis. We also acknowledge the Head, Department of Fisheries and Aquatic Sciences, Prof. Phillip Raburu for availing space in the aquarium lab to carry out this study. We sincerely thank Mr. Andrew Tarus and Ken Rono for sparing part of their busy time schedule to take care of the fish and experimental system. We also take this opportunity to thank Mr. Brunno Cerozi, a Ph.D. student in Aquaponics at the University of Arizona, for discussing the design and giving valuable advice on this study.

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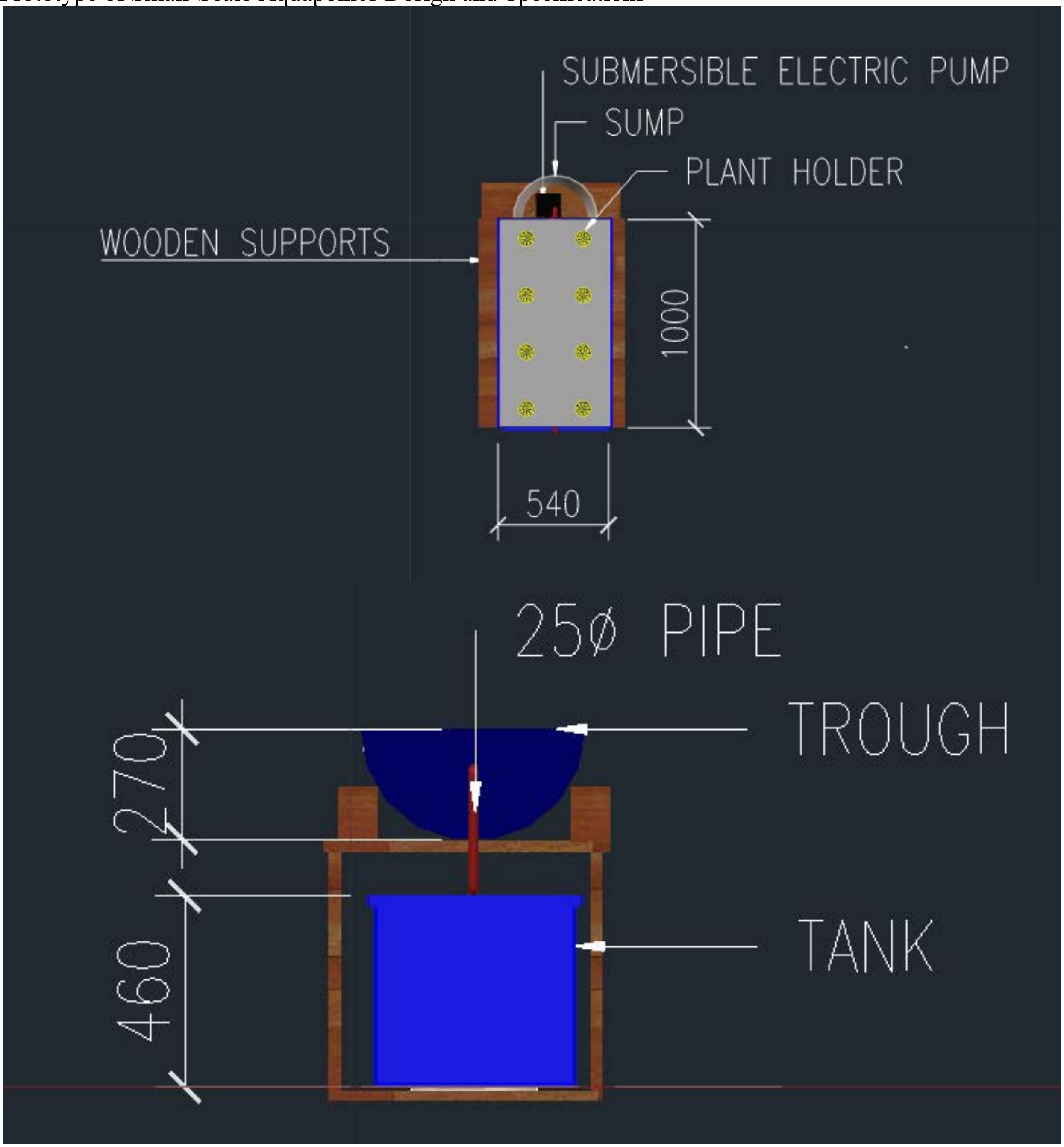
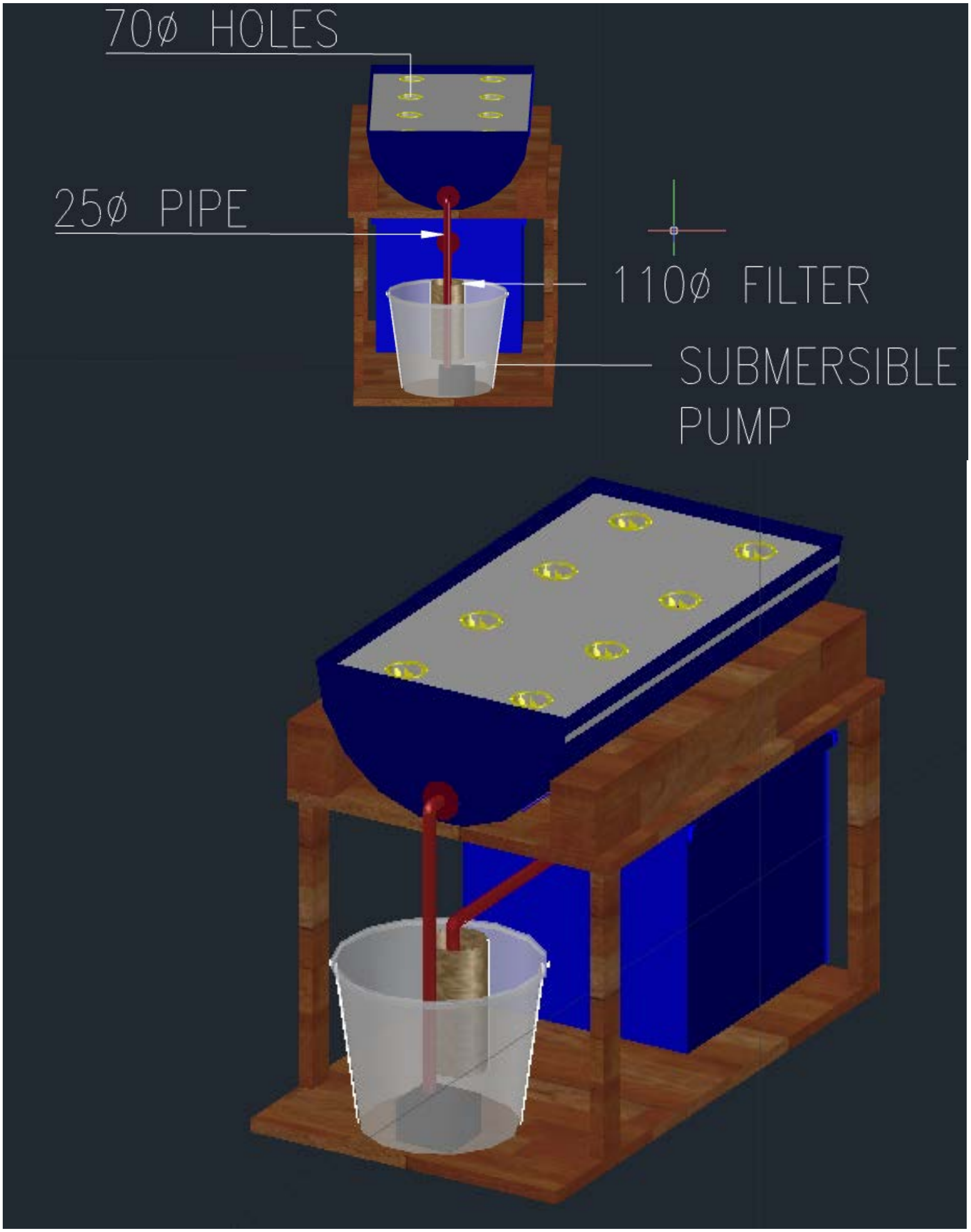
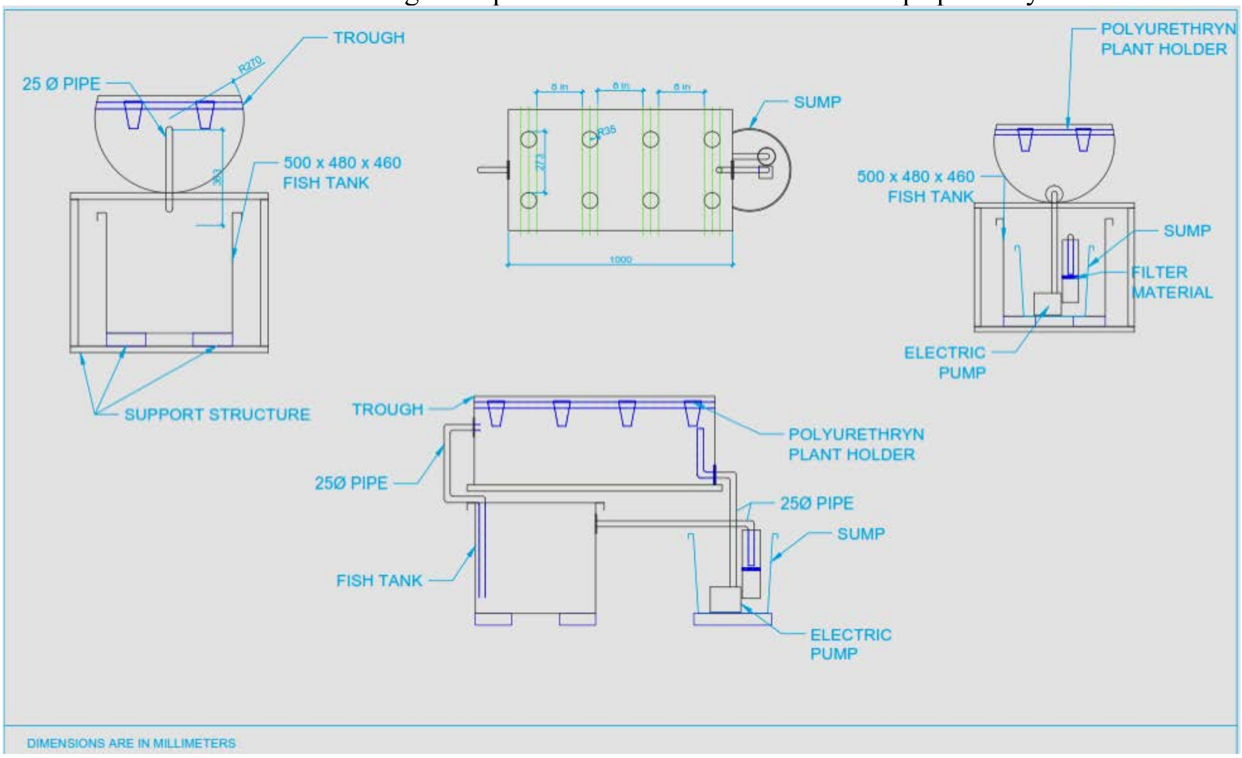
ADDITIONAL FIGURES

Prototype of Small-Scale Aquaponics Design and Specifications




70ø HOLES
25ø PIPE
110ø FILTER
SUBMERSIBLE
PUMP

Cross Sectional Technical Drawing and Specifications of the Small-Scale Aquaponic System



DEVELOPMENT OF LOW-COST AQUAPONIC SYSTEMS FOR KENYA- PART II
DEVELOPMENT AND FIELD TRIAL OF MODERATE-SCALE LOW-COST AQUAPONICS SYSTEM FOR KENYA

Production System Design and Best Management Alternatives/Experiment/13BMA05AU

Julius O. Manyala[1], Kevin Fitzsimmons[2], Charles Ngugi[3], Josiah Ani[1], and Elizabeth Obado[1]

[1]*University of Eldoret, Kenya*
[2]*University of Arizona, USA*
[3]*Kenyatta University, Nairobi, Kenya*

ABSTRACT
A moderate-scale aquaponic system was designed and tested at the University of Eldoret (UoE) fish farm. The objective of designing this system was to demonstrate the concept and training but also offer an opportunity for extension and field trials. The moderate-scale aquaponic designed at the UoE had the capacity of about 200 kg of fish per circular tank and 120 kg fresh weight of vegetables from six floating raft plant beds. The system incorporated a vertical bio-filtration and nitrification unit and a sump operated by gravity flow. All the plant beds drained into a common underground sump through gravity. Complete circulation was achieved by using a primer pump to return purified water into the fish tanks through a gas exchange chamber.

Using trial data from the small-scale aquaponic system, the stocking density of fish and plants were optimized by proportion as well as the flow rate that was regulated by a venture valve to 20 L min-1 to the fish rearing tanks. Field trials were conducted with modified system using African catfish and a mixture of local vegetables, kales and spinach.

Both investment and production estimates were then used to prepare a partial enterprise budget for a single aquaponic system and feed input adjusted by an FCR or 1.5, 1.2 and 1.0 in order to determine the anticipated benefits of the system. Results show that the system can repay a loan within two 6 months growing cycles with a profit margin of 40% to 45% of the initial investment. The hydroponic unit for could be doubled or tripled without affecting the fish ponds due to the use of gravel bed instead of floating rafts.

INTRODUCTION
Many aquaponic systems are known to be efficient in utilization of nutrients (Richard et al. 2008; Wahyuningsih et al. 2015) as well as water conservation but also has its own challenges (Richard et al. 2011). Aquaponic systems are particularly useful in areas with water scarcity. When properly managed, aquaponic systems provide the advantages of both reducing water usage and effluent (Chanagun et al. 2015). Hu et al. (2015) stated that ammonia is firstly oxidized to nitrite by ammonia oxidizing bacteria and then converted to nitrate by nitrite oxidizing bacteria (mainly *Nitrobacter* spp. and *Nitrospira* spp.). Not enough bacteria in this aquaponic system possibly results in deteriorated water while too high bacteria could make fish prone to diseases.

According to Battina et al. (2016), aquaponics technology is applicable to a variety of situations including, commercial, community based urban food production, industrial scale production in rural areas, small scale farming in developing countries or as systems for education and decoration inside buildings. Trials using the University of Virgin Island (UVIA) Aquaponic system have shown that experimental results are usually not attained in field trials for plant yields (James et al. Undated Report).

OBJECTIVES

1. Design a moderate-scale aquaponic system for potential commercial application.
2. Construct a moderate-scale system to develop proof of concept and training.

METHODS

All the materials for design of the moderate-scale aquaponic system was purchased locally from the various hardware retail outlets and assembled at the University of Eldoret Hatchery area. The unit was then assembled within the existing greenhouse to address the issue of low temperatures for fish growth.

The following materials were necessary for assembling the system:

i. Two circular plastic tanks of capacity 10,000 L
ii. Concrete blocks for the plant units and pond liners
iii. Styrofoam sheets
iv. Plastic drum (4) of capacity 100 L each to act as 2 vertical filtration units, a sump for feeding the plant beds and a sump for receiving water from the plant beds and subsequent pumping to a gas exchange chamber
v. Plastic drum of 200L capacity to act as a gas exchange chamber
vi. PVC Pipes of 2" for water reticulation and 4" drain pipe
vii. Primer water pump of at least 6HP, 240V 50-60MHz frequency

The Design

The two circular plastic tanks were installed on a movable concrete base to receive water from the gas exchange chamber and each with an independent overflow into separate vertical filtration and nitrification chambers of 100L capacity each.

Both the vertical filtration units emptied into a common sump that fed the six plant beds through a reticulation system of pipes. Each plant bed was drained independently into a common underground sump from which the water was pumped back into the gas exchange chamber. Water from the gas exchange chamber was designed to flow by gravity. The design removed the necessity to have tow pumps and water flow through both the fish and plant units was by gravity (Figure 1 and 2).

RESULTS

The design specification and layout is shown in Figure1 while a cross sectional view of the moderate-scale aquaponic system is shown in Figure 2. The testing of the system showed a balance of water flow between the fish rearing tanks and the plant beds since pumping of water to the gas exchange chamber was balanced by the gravitational flow through the system.

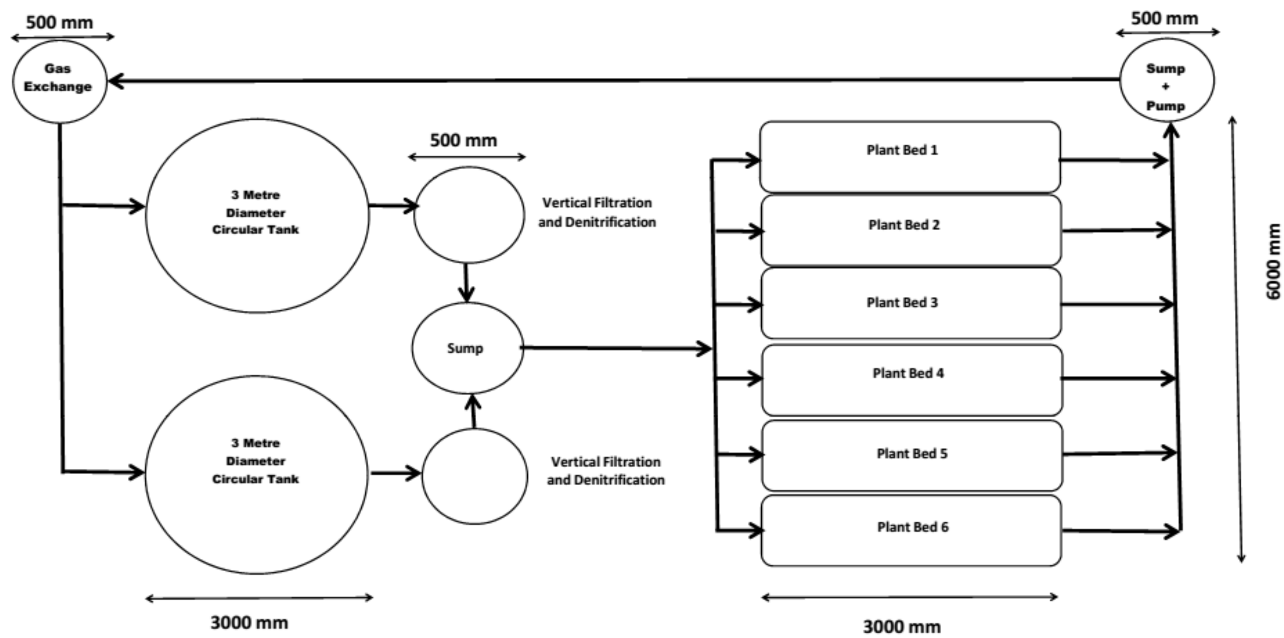


Figure 1. Technical Plan of the Medium-Scale Aquaponic System

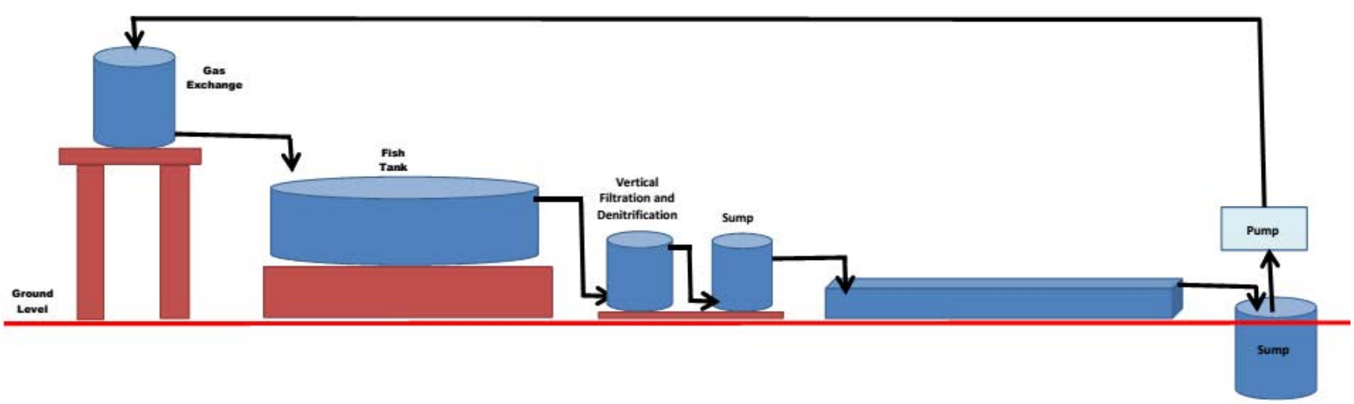


Figure 2. Cross Sectional View of the Medium-Scale Aquaponic System

Plate 1 shows the stages in developing the moderate-scale aquaponic system under when it was under full operation with both fish and plant units functional.

During the development and testing of the moderate-scale aquaponic system, there were five requests from farmers within Eldoret environs to help them develop an aquaponic system. Only one of the lady farmers who had functional ponds and she became a potential candidate for field trials.

Plate 1: i) Top Left-Construction of moderate scale aquaponic system at the University of Eldoret greenhouse, ii) Top Right – Completed moderate-scale aquaponics system, iii) Bottom Left-Nile tilapia in the fish rearing tanks, iv) Spinach in the floating raft plant beds.

Field Trials

Based on the experience from our small-scale floating plant bed, gravel bed and the moderate-scale aquaponic design, the project staff and students assembled at the firm of Robertina Chikamai at Kimumu area, about 2 km firm the University Campus to help her design a practical aquaponic system to integrate the two existing fish ponds with plant beds.

The farm had one large and one medium sized liner ponds of about 500 m2 and 200 m2. She had plans to incorporate poultry farming, strawberry farming and aquaponics. The farmer had initially attempted to install an aquaponic systems but she had challenges in kick-starting the system.

The farmer had opted for gravel bed aquaponic system and since she already had existing ponds, the only logical option was to re-design the system with a pump for the plant beds. We re-designed the system using the bell siphon technology to ensure the hydroponic component doesn't flood unnecessarily but wets the media on which the plants anchor. The crops adopted quickly to the system and had an improved growth in less than 10 days.

The main challenge remains as the cost of recirculating the water due to the cost of electricity. Consequently, the farmer has to periodically switch off the pump. Secondly, running such a pump continuously may damage it unless the pumps are redundant. In view of this challenge, we have contacted our sister department of Physics to assist her in designing a more practical solar powered pump based on existing solar panels in the Kenya market or improvising a wind powered pump for the system. Some of the main highlights of the activities involving aquaponics in the farm are highlighted in Plate 2-4 below:

Plate 2. Harvesting fish from the ponds

Plate 3. Josiah Ani inspecting of the germinating plants

Plate 4. Inspection of the germinating plants

QUANTIFIED ANTICIPATED BENEFITS

We have used the Aquaponic System of Robertina Chikamai to evaluate the potential benefits that are likely to accrue from both fish and plants for a moderate system.

The capital to advance this project was a loan of KES 500,000 ($4,950) provided from the family resources. This amount was used to set up ponds and install the hydroponic component around the ponds. The first pond has approximately a biomass of 1,800 kg of African catfish ready for sale. This translates to KES 540,000 ($ 5,347) at an average price of KES 300 kg-1.

The second pond has been stocked with 1,000 Nile tilapia fingerlings. The fingerlings were bought at a cost of KES 10,000 ($ 99). She has also incorporate poultry farming and strawberry farming in her 0.5-acre farm. The crops have adopted into the system and are doing well. Currently, she is growing a mixture of Traditional vegetables, Kales and spinach. Her vegetables on average give about KES 73,000 ($723) annually from one pond and the hydroponic system. In our opinion, the plant unit could be doubled or tripled for every aquaponic system by adding another row of plant growing beds.

From the trials, we have attempted to carry out a partial enterprise budget to illustrate the anticipated benefits from the aquaponic system based on three Food Conversion Ratios (1.5, 1.2 1nd 1.0) based on a single aquaponic system and based on African catfish production. Factoring the FCR is critical in environments such as Kenya where feed is still a major constraint in terms of availability, quality and quantity.

Table 1. Anticipated benefits of aquaponic system based on catfish, local vegetables, kales and spinach at the Robertina Fish Farm and FCR of 1.5

	UNITS	QTY	UNIT PRICE	KES	US $
Loan				500,000	4,950
Pond construction	ITEM	1	25,000	25,000	248
Catfish Fingerlings	PCS	1,000	10	10,000	99
Feeds (Assuming FCR of 1.5)	KG	2,700	100	270,000	2,673
Labour	MONTHS	6	10,000	60,000	594
Electricity	MONTHS	6	3,000	18,000	178
			Sub-total	383,000	3,792
Incidentals (12% of Total costs)	ITEM		**Incidentals**	45,960	455
			Total Costs	**428,960**	**4,247**
Potential Fish Harvest					-
Catfish	KG	1,800	300	540,000	5,347
Potential Vegetables Harvest					-
Local	KG	600	200	120,000	1,188
Kales	KG	600	100	60,000	594
Spinach	KG	600	100	60,000	594
			Total Income	**780,000**	**7,723**
			Net profit	**351,040**	**3,476**

Table 2. Anticipated benefits of aquaponic system based on catfish, local vegetables, kales and spinach at the Robertina Fish Farm and FCR of 1.2

	UNITS	QTY	UNIT PRICE	KES	US $
Loan				500,000	4,950
Pond construction	ITEM	1	25,000	25,000	248
Catfish Fingerlings	PCS	1,000	10	10,000	99
Feeds (Assuming FCR of 1.2)	KG	2,160	100	216,000	2,139
Labour	MONTHS	6	10,000	60,000	594
Electricity	MONTHS	6	3,000	18,000	178
			Sub-total	329,000	3,257
Incidentals (12% of Total costs)	ITEM		**Incidentals**	39,480	391
			Total Costs	**368,480**	**3,648**
Potential Fish Harvest					-
Catfish	KG	1,800	300	540,000	5,347
Potential Vegetables Harvest					-
Local	KG	600	200	120,000	1,188
Kales	KG	600	100	60,000	594
Spinach	KG	600	100	60,000	594
			Total Income	**780,000**	**7,723**

	UNITS	QTY	UNIT PRICE	KES	US $
			Net profit	**411,520**	**4,074**
Loan				500,000	4,950
Pond construction	ITEM	1	25,000	25,000	248
Catfish Fingerlings	PCS	1,000	10	10,000	99
Feeds (Assuming FCR of 1.0)	KG	1,800	100	180,000	1,782
Labour	MONTHS	6	10,000	60,000	594
Electricity	MONTHS	6	3,000	18,000	178
			Sub-total	293,000	2,901
Incidentals (12% of Total costs)	ITEM		**Incidentals**	35,160	348
			Total Costs	**328,160**	**3,249**
Potential Fish Harvest					-
Catfish	KG	1,800	300	540,000	5,347
Potential Vegetables Harvest					-
Local	KG	600	200	120,000	1,188
Lkales	KG	600	100	60,000	594
Spinach	KG	600	100	60,000	594
			Total Income	**780,000**	**7,723**
			Net profit	**451,840**	**4,474**

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Development of Low-Cost Aquaponic Systems for Kenya- Part III
ASSESSMENT OF THE IMPACT OF ECONOMIC STIMULUS PROGRAMME ON SUPPLY ENHANCEMENT, RURAL POVERTY ALLEVIATION AND FOOD SECURITY

Production System Design and Best Management Alternatives/Experiment/13BMA05AU

Julius O. Manyala[1], Kevin Fitzsimmons[2], Charles Ngugi[3], Josiah Ani[1], and Elizabeth Obado[1]

[1]*University of Eldoret, Kenya*
[2]*University of Arizona, USA*
[3]*Kenyatta University, Nairobi, Kenya*

ABSTRACT

This report covers the stakeholders' consultation at county level on the impact of Economic Stimulus Programme (ESP) implemented by the Government of Kenya on fish farming from 2007 to around 2012 when Kenya adopted a devolved Government System and aquaculture became a function of the County Governments. A number of counties selected for assessment included: a) Kisii; b) Vihiga; c) Kakamega; d) Bungoma; e) Nyeri and f) Meru Counties between 21st August and 6th September 2016. A total of 59 stakeholders were engaged on discussion on fish farming input availability and types such as feeds, seeds, pond liners, fishing gear, chemicals (hormones) and other piping/plumbing materials for construction of fish holding facilities. Further assessment was conducted on supply enhancement, rural poverty alleviation and food security. Diverse production systems were found to exist in Kisii, Vihiga and Kakamega while Bungoma had mainly of earthen ponds. In Kakamega, Nyeri and Meru, there was a mixture of earthen ponds and liner ponds due to the porosity of the soils. In addition, there are a number of large dams (up to 20 Ha) that have been variously stocked by the fisheries department to enhance production, income, protein availability and employment to the riparian communities. All these dams have management committees in place to regulate all the socio-economic activities related to water resource use and management.

There are over 6 large-scale fish feed producers in Kenya and numerous small-scale fish feed manufacturers. In addition, many farmers have resorted to on-farm feed manufacture to reduce to cost of feeds in their farms. There is a considerable amount of fish feeds imported into Kenya by Aller Aqua, Ranan Feeds and Skretting. The ESP, however, assisted in developing small-scale feed production units across all the 210 counties targeted by the program. Most of the small-scale feeds produced by farmers are sinking pellets as opposed to the preferred extruded and floating pellets from large-scale manufacturers. The feed availability was found to be adequate but the quality varies considerably between both small-scale and large scale manufacturers.

The ESP trained a number of local farmers in the production of monosex tilapia and over 30 hatcheries were registered by the Government to provide tilapia seeds to farmers. These hatcheries still exist but the demand for monosex fingerlings have declined considerable with the termination of subsidies from ESP.

The survey revealed that the current fish production from aquaculture has declined considerably due to the abrupt termination of the Government subsidies implemented during the ESP and Fish Farming and Enterprise Productivity Programme (FF&EPP). This abrupt termination was occasioned by the implementation of the new Kenyan Constitution in 2012 that devolved aquaculture function to Counties. The aquaculture sub-sector is characterized by weak marketing structures and hence low income since most of the fish are sold at the farm gate without much preservation, processing or value addition.

A few farmers in Kakamega and Meru specifically deep fry some of their fish produce for sale in their retail outlets. In Kisii County, the retail outlets is slightly well developed with ice boxes and motorcycles for collecting fish from farmers and processing facilities for production of fish sausages, samosas and minced fish products. The implementation of ESP is thought to be a major factor in enhanced availability of cultured fish and hence both food and nutrition security in many parts of rural Kenya.

To improve on fish marketing, the Government constructed four mini processing plants in Rongo, Kakamega, Nyeri and Meru with blast freezers, cold storage and ice plants. In addition, collection centers were created and each one of them equipped with chest freezers with a capacity of about 200 kg per day. So far, only the Nyeri plant is operational and uses a refrigerated truck to collect fish from the collection centers. The Nyeri plant was operationalized by a grant-in-aid of KES 2,400,000.00 (approximately US $ 240,000) from the County Government of Nyeri through the Kenya Agricultural-Sector Productivity Programme (KAPP). The farmed fish value chain seems to be complete but with weak linkages, support services, technological innovations, asset financing, credit lines and product development among other factors. All the counties visited had existing plans that currently offer subsidies to fish farmers in terms of fingerlings and feeds. There are also plans to construct hatcheries within the County Integrated Development Plans (CIDP) but many of these plans have not been implemented due to inadequate funding and budgetary constraints.

The most active and prominent farmer organization with a national scope is the Aquaculture Association of Kenya (AAK) which has been active in mobilizing fish farmers in capacity building and value addition. AAK has sub-branches in almost all the counties with high aquaculture potential and has registered growing membership. The AAK is currently undertaking activities in capacity building in value addition through a grant from the United Nations International Development Organization (UNIDO). Other programmes that have supported aquaculture in the past in Kenya include: a) Kenya-German-Israel Trilateral Project; b) FarmAfrica; c) Food and Agriculture Organization (FAO) African Sustainable Trust Fund (ASTF) targeting specifically Coordinating Unit (ASCU); and d) CRSP in Pond Dynamics e) Aller Aqua on training and feed formulation. A number of issues that require addressing include technology transfer, certification, financing mechanism of aquabusiness and policy matters.

Background Information

Prior to the year 2007, several initiatives on fish farming in Kenya had been executed by the Department of Fisheries, The main activities were geared towards using fish farming as a tool for poverty alleviation and food security, and were addressed through various project activities that included but not limited to: pond construction and management, stocking rates trials, feed trials, integration of fish farming with other agricultural activities, brood stock management, seed quality and evaluation of growth performance of Nile tilapia and African Catfish.

To enhance aquaculture production, the State Department trained fishers, implementing officers and stakeholders on fish farming practices; conducted a national aquaculture suitability appraisal and developed suitability maps for the 210 Constituencies; developed a fish breeding structure with a holding capacity of over 200,000 brood-stock; developed fish feed specifications for tilapia, catfish and trout and related supply chain; procured 54 Fish Feed Pelletizing machines and distributed them to the constituencies; procured 148 Motorcycles and recruited 286 Fisheries Extension Officers for extension service delivery in the constituencies; constructed (4) Fish Processing Plants in Tetu, Imenti South, Rongo and Lurambi constituencies; constructed a state of the art fish processing factory in Mitunguu, Meru County in collaboration with private sector investors; constructed 3 Recirculation Aquaculture Systems (RAS) in Kiambaa (Jambo Fish Farm & Samaki Tu Fish Farm) and Kisumu

Rural (Thinqubator Fish Farm) Constituencies; constructed over 69,998 fish ponds country-wide (46,824 fish ponds in 160 Constituencies country-wide by GoK, and some other 23,174 ponds under the multiplier effect by farmers & investors and stocked them with over 100 million fingerlings; increased the area under aquaculture from 722 Ha to 2,105.1 Ha; increased national aquaculture production from 4,220 MT to 23,501 MT; and created direct employment for over 100,000 fish farmers, short-term employment for over 100,000 youths and indirect employment for over 500,000 other Kenyans along the aquaculture value chain.

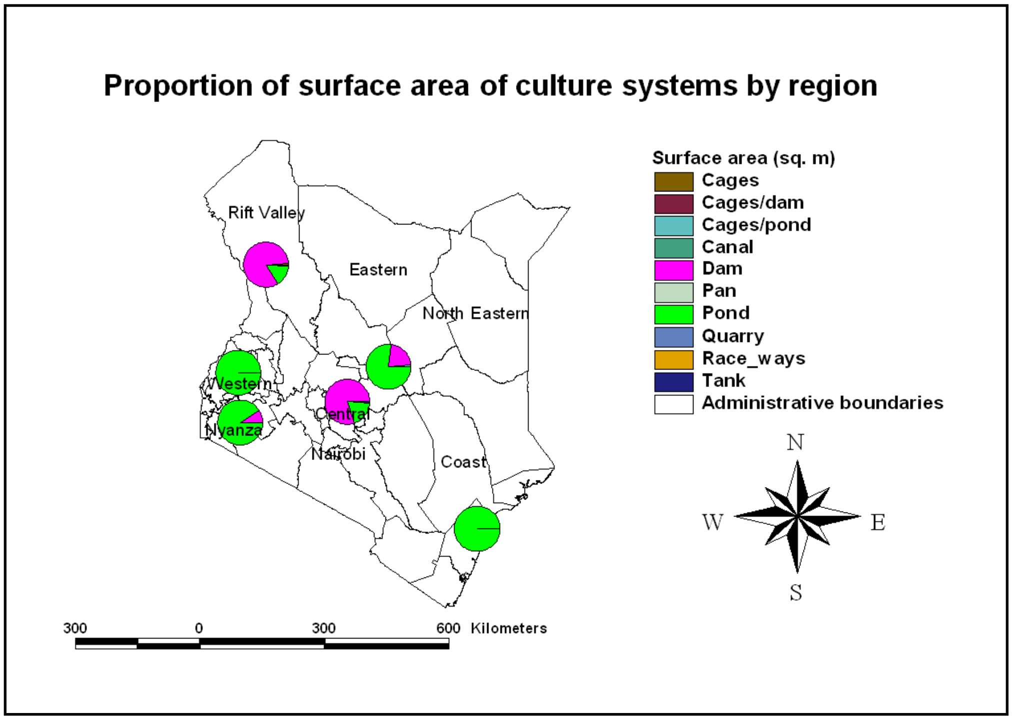

Figure 1. Map of Kenya showing the proportion of surface areas (m2) of aquaculture facilities by region; Coast, Eastern, Central, Rift Valley, Western and Nyanza (Source: Ngugi and Manyala, 2009).

The Initiation of the Fish Farming Economic Stimulus Programme started during the 2009/2010 financial year in Kenya, was envisaged to revolutionized fish farming practices in the country and has make Kenya a fish producing and fish eating nation. The project was implemented in high aquaculture potential areas of Western Kenya, Nyanza, parts of Rift Valley, Eastern, Central Kenya and Coast regions. These regions are endowed with suitable water resources that include springs, wetlands, rivers, water reservoirs and the temporary water bodies.

The State Department of Fisheries focused on promoting aquaculture development in the country to counter the declining production from capture fisheries. Aquaculture, being a food production subsector, was seen as an avenue and opportunity to contribute towards food security, generate income and create employment to rural communities, especially women and the young generation.

There has been an apparent progressive increase in farmed fish production based on the Fisheries Statistical Bulletins (GoK, 2006; 2008; 2010; 2012; 2014). Fish farming production during the year (2013) was estimated at 21,486, 828 kg (21,487 metric tons) with a farm gate value of KES 4,633,634,405 compared to 19,584,843 kg (19,585 metric tons) valued at KES 4,223,471,393 in 2011. Out of the total farmed fish production, Nile tilapia contributed 75% (16,115 metric tons), African catfish 18% (3,868 metric tons), Common carp 6% (1,289 metric tons) and Rainbow trout 1% (214 metric tons). This production was from 68,734 ponds with an area of 20,620,200 square meters

(2,062 hectares), 161 tanks measuring 23,085 square meters and 124 reservoirs with an area of 744,000 square meters throughout the country. Over the last ten years, fish production has increased from as low as 1,012 metric tons produced in year 2003 to the present production of 21,487 metric tons, figure 2.

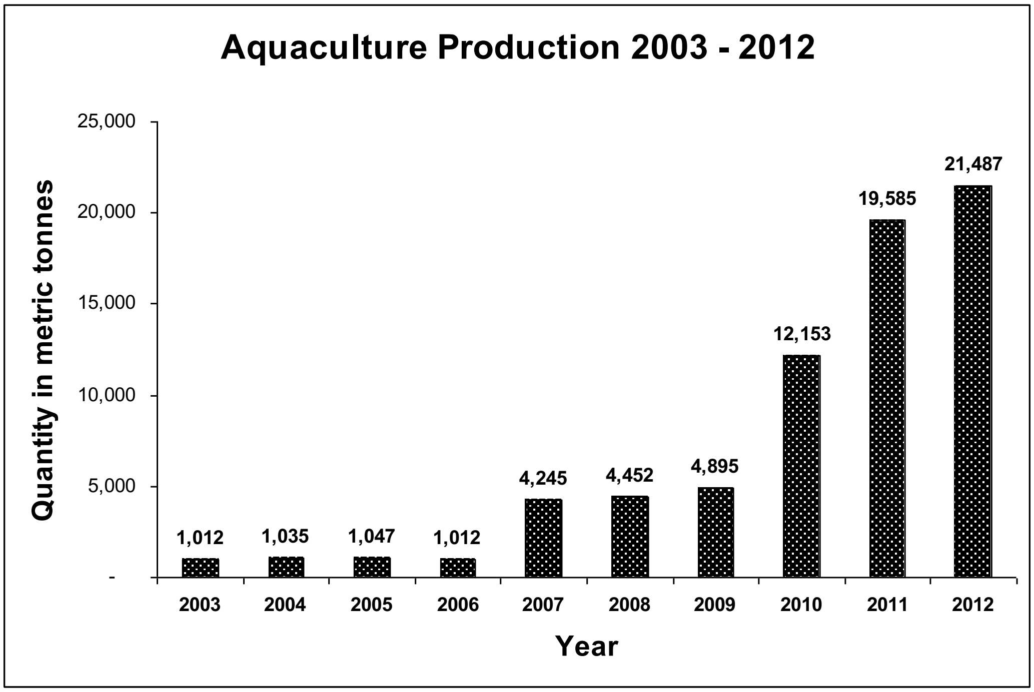

Figure 2. Aquaculture production for last ten years (2004-2012) showing the exponential increase in culture fish production in 2010 attributed to ESP

Over time, the following constraints have been identified to affect aquaculture activities in Kenya:

i. Lack of readily available and affordable quality fish seed (fingerlings);
ii. Lack of adequate good quality and affordable fish feeds;
iii. Poor adoption of fish husbandry techniques by some farmers even after being trained on basic pond management;
iv. Water scarcity due to other competing uses – industry, domestic and agriculture;
v. Lack of and /or inadequate accurate market information for use by fish farmers;
vi. Lack of good credit facilities and schemes for fish farmers;
vii. Security and safety of fish in ponds posed by thieves and predators;
viii. Poor book keeping and record management leading to inaccurate data from farmers along the aquaculture value chain e.g. input costs, management cost, quantities of fish harvested and value;
ix. Sub optimal staffing levels especially extension personnel;
x. Inadequate facilitation in terms of transport and timely funds towards carrying out of fisheries extension service provision.

Objective 4 of the study considered the impacts of the Kenya aquaculture stimulus project that aimed at constructing 200 farming ponds for 140 constituencies. The government effort expanded fingerling production, subsidized fingerling distribution, and endeavored to enhance technical assistance to producers. The present survey focused on the status of input supply for aquaculture, production and production trend, marketing, development plans farmer organization as a backstop for poverty alleviation and food security in the rural economy. The report outlines past interventions in relation to ESP and their impacts in the development of aquaculture in Kenya in the context of capacity building.

OBJECTIVE

Assess the government funded Economic Stimulus Programme impacts on fish farming in terms of supply enhancement, rural poverty alleviation, and food security

MATERIALS AND METHODS

The assessment involved several steps that included:

1. Mobilization of the stakeholders through the Intergovernmental Secretariat (IGS) and the Council of Governors (CG) through a formal request to the State Department of Fisheries (SDF)
2. Identification of contact persons in each county to facilitate meetings and field visits.
3. Development of information collection tools:
    a. Key Informant Interviews (KII) checklist
    b. Structure questionnaire
4. Stakeholders engagements, consultations and field visits
5. Compilation of the assessment of the impact of ESP on inputs supply, poverty alleviation and food security among other socio-economic parameters.

RESULTS

The findings from the stakeholders' analysis could be summarized into the following sub-sections:

Input Supply- *Feeds Supply:*

In all the Counties visited, the Economic Stimulus Programme (ESP) and Fish Farming and Enterprise Productivity Programme (FF&EPP) were instrumental in providing farmers with fish feeds from 2009 to 2012. The main feeds suppliers identified by the stakeholders during the consultation were: a) Dominion Farms Ltd.; b) Jewlet Fish Farm; and c) Sigma Feeds. In Vihiga and Bungoma County, some fish farmers bought seeds from Bidii Fish Farmers in Luanda (Emuhaya).

The ESP and FF&EPP also provided the extension services for construction of earthen ponds to many fish farmers as well as technical advice. During this period, there was a considerable rise in fish production from aquaculture, apparently stimulated by the subsidies.

Plate 1. i) Top Left-Large scale feed producer from Bungoma (Eden Millers), ii) Top Right-Range of feed products offered by retail outlet Sweetex, iii) Bottom Left-Small-scale feed stockiest (Sweetex Animal Feeds), iv) Bottom Right-Imported fish feeds (Aller Aqua)

Seeds supply:

In all the Counties already visited, the Economic Stimulus Programme (ESP) and Fish Farming and Enterprise Productivity Programme (FF&EPP) were also instrumental in providing fingerlings to farmers from 2009 to 2012. The fingerling suppliers frequently mentioned by the stakeholders were; a) Dominion Farms; and b) Jewlet Fish Farm; c) Sagana National Aquaculture Research and Development Centre; and d) Lake Basin Development Authority (LBDA).

Plate 2. i)Top Left-Circular concrete nursery tanks for monosex tilapia fingerlings at Tigoi Fish Farm, ii) Top Right-Rectangular concrete nursery tanks for monosex tilapia fingerlings at Tigoi Fish Farm

During this period, a number of Private Sector (PS) operators were contracted by the Government to supply both fish feeds and fingerlings to farmers and all expenses were met by the programmes. A number of hatcheries were subsequently established by Private Sector (PS) operators to meet the high demand for fingerlings in the country in addition to the Government operated hatcheries.

The emphasis of ESP and FF&EPP were on monosex tilapia and this preference technically attributed to faster growth and better yields as compared to mixed sexes. All the Counties visited are still providing fish farmers with monosex tilapia as a subsidy to fish farming from the same sources as the ESP/FF&EPP. The scale of subsidy to fish farming has gone down considerably and all supplies are purchased through the established procurement procedures at the county level.

All the counties visited have in place some budgetary allocation for the whole Ministry of Agriculture, Livestock and Fisheries and the financing is shared between all the directorates. The budgetary allocation for fish farming in all the counties seem to be inadequate to provide effective service delivery to fish farmers in terms of seeds and feeds.

Other inputs:
The construction costs were borne by the programme and a number of trained individuals were contracted to by the Government to construct earthen ponds for farmers. The basic ponds piping and fittings were also provided free of charge to the farmers.

With the advent of private hatcheries and operators, there arose further requirements to import hormones for sex reversal and hapa nets for nursery management of fingerlings. The demand for ethanol also increased considerably for monosex tilapia production. Since ethanol is a classified chemical, special authorization is required to purchase it under.

Production- ***Production Systems:***
The ESP and FF&EPP facilitated the construction of earthen ponds and a limited number of liner ponds. Pond lining was used as a strategy to mitigate water leakages and seepages in ponds where soil types were not suitable for pond construction.

In Kisii, Vihiga, Kakamega and Bungoma, most of the culture facilities consist of earthen ponds because the soils are suitable for construction of such ponds. However, in Nyeri and Meru, a number of farmers use liner ponds as well as raised ponds due to the porous nature of the soils in these regions. In both cases, the cost of setting up these culture systems were borne by the ESP and FF&EPP.

Plate 3. i) Top Left – Pond culture in Bungoma County, ii)Top Right-Expansive pond culture system in Vihiga County, iii) Bottom Left- Pond system in Kakamega County, iv) Bottom Right-Aquaponic System at Tigoi Fish Farm (Vihiga County)

Trends in production:
Tremendous increase in production was attributed by all the stakeholders consulted on the input supply provided by the Government from 2008 to 2012. The production declined considerably thereafter due to two reasons: a) The new Kenya Constitution after 2012 General Elections provided for a two tier devolved government system with shared or devolved functions at each level; b) aquaculture became a devolved function under the County Government; and c) there was no exit strategy as the National Government could not continue to fund aquaculture through the ESP/FF&EPP.

The current state of fish farming indicate that: a) More than half of the ponds and facilities constructed under the ESP and FF&EPP have not been re-stocked after harvesting; b) There is limited input supply, especially of feeds to farmers either through subsidy or personal efforts; c) Extension services are limited; and d) production has generally declined in the last 4 years.

Marketing
The marketing structures for farmed fish is highly variable and at different levels of development among the counties. In Kisii County most of the fish is sold at the pond side (farm gate) but there are a couple of retail outlets developed by the ESP and FF&EPP. The retail outlets are equipped ice boxes and motorcycles to collect fish from farmers for processing, preparation and sale. The shops are equipped with facilities for preparing fish sausages, samosas and fish balls.

Plate 4. i) Left and Centre – Modern food processing equipment used for fish value addition in Kisii County, ii) Top Right-Value added fish products in Kisii County, iii) Bottom Right- Display unit for value added fish products in Kisii County

Poverty Alleviation
In Vihiga, the County Government invested in a retail outlet in each of the four sub-counties where the shops were renovated and equipped with chest freezers as retail outlets. So far, only one of them is operating at Emuhaya while the rest are closed due to inadequate supply of fish. Kakamega County has a mini-fish processing plant which is un-operational and hence the fish is sold at the farm gate or rarely transported to local markets for sale. One fish farmer is known to transport fish to Nairobi using public transport and one group has established a retail outlet at Navakholo with a capacity of

200 Kg at a time. Some farmers undertake processing by deep frying before selling the fish but all these are informal operations.

Plate 5: Shelter (house) constructed entirely from proceeds of fish farming and inset showing diversification and acquisition of high yielding dairy animals from proceed of fish farming by the same fish farmer.

In Bungoma County, there is no specific marketing structure or strategy and most of the fish is sold at farm gate. Some of the fish is transported to local markets and sold alongside the wild caught fish but faces stiff competition, especially from imports from Uganda through Malaba border.

Food Security

During the ESP/FF&EPP, the Government Constructed four mini fish processing plants in Nyeri, Meru, Kakamega and Rongo. These plants were equipped with blast freezers, cold storage facility and ice -making plants in order to address the issues of marketing. Only one plan in Nyeri is operational and is being managed by the Fish Farmers Co-operative Society though the plant is operated by staff from the County Directorate of Fisheries due to inadequate technical capacity by the Co-operative Society in fish processing and quality assurance. The Society has acquired a refrigerated truck for collecting the fish from designated fish collection centres. Additionally, the fish collection centres are equipped with deep freezers to keep the fish fresh before collection.

The Nyeri mini fish processing plant has been operationalized by a grant-in-aid of KES 2.4 million from the County Government. To guarantee adequate fish supply, the county has also invested in re-stocking of fish ponds of more that 130 farmers with 1,000 tilapia fingerlings and earmarked KES 2,200,000 for re-stocking in the 2016/2017 financial year.

Plans are underway to hand over the Meru and Kakamega minis fish processing plants to the Fish Farmers Co-operative Societies to manage. The marketing model being adopted by the Meru Fish Farmers Co-operative Society in running the mini-processing plant is shareholding by the fish farmers. The society intends to collect fish from Nyeri, Kirinyaga and Embu to meet its operational capacity as it awaits increased production from its own farmers.

Plate 6: Modern fish processing plant in Nyeri County for processing and marketing of farmed fish

Development Plans
Since the termination of ESP following the implementation of the 2010 Kenya Constitution, the function of fish farming and aquaculture is entirely devolved to the County Governments. In order to guarantee increased effort and investment in the aquaculture sub-sector, the County Governments are expected to streamline aquaculture activities in teir development plans as well as make budgetary provisions for promoting and supporting aquaculture activities. A sample review of the existing Aquaculture Development Plans for Kisii, Vihiga and Bungoma is briefly presented in the following section.

Kisii County
The County has earmarked the development of three fish multiplication centres for fingerlings production, training and a demonstration facility. One of these facilities is under construction and near completion, having been allocated funds for operationalization in the County Integrated Development Programme (CIDP) in 2016/2018 financial years while another two are earmarked for development in future. It is planned that each sub-county will eventually have at least one such centres in future. The county also plans to construct a fish cold storage facility as part of the municipal market.

Vihiga County
The County took over the Mitoko Fish Farm from the National Government and is developing it as a training centre a hatchery to provide fingerlings to its farmers. The county has been subsidizing fish farmers with both fingerlings and feeds as part of its regular extension service. It is proposed in the CIDP to implement a capacity building programme in fish farming throughout the county in the 2016/2018 financial years.

Bungoma County
The County has plans to develop a trout hatchery in Mount Elgon to supplement the tilapia and catfish farming activities in the county. The County also allocated resources in the El Nino fund to rehabilitate fish ponds and supply input to fish farmers under the agricultural sector interventions in the 2017/2018 financial year.

Farmer Organizations

Prominence of Fish Farmers Cooperative Society has been recorded in Kakamega, Nyeri and Meru and all of them have an objective in marketing. The marketing of farmed fish has been hampered in almost all the counties by lack of marketing infrastructure. Since mini processing plants were developed in Rongo, Kakamega, Meru and Nyeri, the production has not been adequate to run these plants except in Nyeri (at almost half capacity).

The biggest challenge that is currently being faced by the farmer organizations is how t increases production to a level where it becomes economically viable to embark on developing and investing on marketing infrastructure. At the moment, there are no fish farmer organizations that provide financial services or asset financing to its members.

The Aquaculture Association of Kenya (AAK) has been instrumental in capacity building and organizing fish farmers into networks for purposed of value addition interventions. AAK has had some funding from United Nations International Development Organization (UNIDO) to support its activities. AAK is currently operating as branches in most counties with high potential in fish farming.

Past and Present Interventions

There has been a number of interventions in fish farming in Kenya dating from 1950s from the American Peace Corps but the most recent in the last five years include: a) Government supported Economic Stimulus Programme (ESP) and Fish Farming and Enterprise Productivity Programme (FF&EPP); women and youth; c) FarmAfrica Capacity Building programme through the Agricultural Sector b) Food and Agriculture Organization (FAO) African Sustainable Trust Fund (ASTF) targeting specifically Coordinating Unit (ASCU); d) Aller Aqua training on feed formulation; and e) Kenya-German-Israel Trilateral Project on fish farming involved in tilapia fish value chain and capacity building as the main programmes

The Collaborative Research Support Program (CRSP) and AquaFish Innovation Lab has been operating in Kenya for a total of almost 25 years in research capacity building and field trials. The program has produced some of the custom extension information and materials presently being used in the aquaculture industry in Kenya. In addition, many of the beneficiaries of this programm are actively involved in aquaculture enterprises in Kenya.

Existing Farmed Fish Value Chains

The existing farmed fish value chains consist of the following input supplies: a) fingerlings (seeds); b) feeds and feed ingredients; c) various chemicals including hormones for sex reversal; d) fishing gears; e) pond liners and fittings and f) packaging materials for fingerlings; g) brood stock for hatchery operators

The services required in the aquaculture industry include: a) ponds construction; b) extension; c) capacity building and training d) networking and technology services. The transformation and logistics required in the value chain include: a) chilling and freezing; b) processing; c) transportation; and d) cooling facilities. Value addition is part and parcel of marketing, sales and consumptions. The development of products was found to be poor during the stakeholders consultation with most of the fish being marketed whole and fresh but without the necessary infrastructure.

DISCUSSION

In view of the current weak policy framework for aquaculture development in Kenya, there are a number of issues that can be addressed from both technical and policy perspectives. Some of these issues include financing mechanisms for aquabusiness, certification in aquabusiness and policy reviews and amendments to address emerging issues such as cage culture in natural water bodies among others.

Aquaculture financing and development

It is clear from the existing information the development of aquaculture in Kenya has been slower than expected due to lack of inputs and financial partnerships. Since there are new prospects of financing aquaculture projects be micro-financial institutions and commercial banks such as Equity, this development should be streamlined into the Aquaculture Policy by the Ministry responsible for Fisheries.

However, there could be other major players in the financial sector that include the Treasury and Central Bank in Kenya that have statutory control over financial institutions in Kenya. The inclusion of these relevant institutions in drawing the policy would ensure that there are provisions of exemptions for inputs wherever required and whenever possible as applicable in many agricultural sectors in Kenya.

Developing private-public partnerships is an option that is currently gaining popularity in the development agenda in Kenya. Several options are available to foster this option but there are no guidelines on how this can be achieved.

There is need to involve and encourage nascent young and growing commercial aquaculture producers or community aquaculture development project in joint funding applications for research in collaboration with academic and research institutions such as University of Eldoret, Egerton University, Kisii University, Maseno University and KMFRI all of which have both human and infrastructure facilities for aquaculture and aquatic sciences. Some past and successful interventions require up-scaling such as USAID-KBDS Baitfish Cluster Development. This approach will guarantee not only positive research findings on key constraint to production and marketing but also for a constructive partnership between researchers and producers and improve needs driven capacity of research institutions.

Partner with large scale commercial fish farmers through production agreements in the form of out-growers such as practiced in the tea, sugarcane and some rice schemes in the country. This approach requires that contracted out-growers are provided with inputs at a cost and the cost is recovered at the time of delivery. The large scale farmer would need a business plan for financing this approach and this could be a possible source under long-term investment plans other than a simple business plan.

Some of the existing trust lands could be allocated to existing development agencies on request for the express purpose of aquaculture. This would require a policy framework and involvement of the Ministry of Lands (Commissioner for Land).

Some existing Government facilities that are essentially used as demonstration centres could be upgraded into commercial farm level by a group of entrepreneurs so that they run the farms on a commercial basis or on lease. There would be several conditions to fulfill such as developing a business plan and obtaining financial security for such an undertaking. This would guarantee that the centres are used for the intended purpose of demonstration but the emphasis shifted to large scale commercial production i.e they pay for themselves and eventually become economically sustainable. These facilities could be run on partnership with government agencies for research, extension and production.

Aquaculture certification
There is no clear certification process, procedure or implementation in aquaculture in Kenya. Even though the Government has developed a number of standards for fish feeds and fish quality through its national institution, the Kenya Bureau of Standards (KEBS), not even a single aquaculture enterprise has been certified yet. The structure for certification exist through the Kenya Accreditation Services (KAA) but it is hard to find any suitable Conformity Assessment Bodies (CAB) for aquaculture in the region.

Kenya Accreditation Service (KENAS) is the Sole National Accreditation Body (NAB) mandated to offer accreditation services in Kenya. It is established under the States Corporations Act, Cap 446; vide Legal Notice No. 55 of May 2009. KENAS gives formal attestation that Conformity Assessment Bodies (CABs) are competent to carry out specific conformity assessment activities. A CAB is a testing laboratory, calibration laboratory, certification body or an inspection body that provides inspection, testing, and certification services in all fields in the public and private sectors.

Aquaculture policy
Even though the draft National Fisheries Policy has a section on aquaculture, it is necessary to develop an aquaculture policy in parallel to a more general fisheries management policy. An aquaculture policy is specifically necessary because it will directly address issues of food security in line with the Strategy for Revitalization of Agriculture (SRA). Usually, the Ministry responsible for fisheries is responsible for developing such a policy but it is desirable that a very wide stakeholder's consultation is carried out during the policy development. The Aquaculture Policy will address a wide range of issues including the new development of cage culture in natural water bodies (sea ranching), certification and investment plans. This policy will not only address policy concerns but also provide a framework for stimulating rapid development in aquaculture by recognizing the critical input sector, technological sector, extension and marketing. The policy can possible be prepared in 3-5 years with both government and development partner funding.

Once the aquaculture policy is put in place, there would be need to harmonize various sections of legislation to avoid overlap, contradictions and conflicts. For example, export of aquarium fish is subject to live fish movement permit and aquarium fish dealers license under the current Fisheries Act while when it comes to certification for export, it is the veterinary department who is responsible.

The Public Health Act and the Environmental Management and Coordination Act (EMCA) are already in conflict when it comes to wetlands, standing pools of water and their utilization. While an envisaged aquaculture policy and act would encourage the development of standing waters (ponds and other facilities) for fish farming, the Public Health Act considers these as a health nuisance and hazards that should be drained and disinfected and EMCA prohibits the use, drainage or utilization of wetlands for either personal or commercial purposes.

Human resource (Extension)
Extension has been identified as one of the constraints in aquaculture development, it would be appropriate to consider strengthening this area by:

i. Formation of target groups and farmer-to-farmer clusters with the ultimate goal of developing a critical mass of fish farmers who are able to move aquaculture to commercial level.
ii. Organizing field days for farmers with demonstration centres for better technology transfer
iii. Training clusters of fish farmers in aqua-business in line with the upgrading of demonstration centres into full scale production centres through various partnerships.

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TOPIC AREA

SUSTAINABLE FEED TECHNOLOGY AND NUTRIENT INPUT SYSTEMS

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PELLET FEED IMPROVEMENTS THROUGH VITAMIN C SUPPLEMENTATION FOR SNAKEHEAD CULTURE

Sustainable Feed Technology and Nutrient Input Systems/Experiment/16SFT01UC

Tran Thi Thanh Hien[1], Pham Minh Duc[1], Nguyen Van Khanh[1], Tran Thi Tuyet Hoa[1], Tran Minh Phu[1], Chheng Phen[2], Phanna Nen[2], Marta Gomez-Chiarri[3] and David A. Bengtson[3]

[1]College of Aquaculture and Fisheries, Can Tho University, Vietnam
[2]Inland Fisheries Research and Development Institute, Cambodia
[3]Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Kingston, RI, USA

ABSTRACT

Vitamin C, or ascorbic acid (AA), is important in growth and physiological functions of fish. Six soybean meal-based (SBM) diets containing 0, 125, 250, 500, 1000 and 2000 mg.kg^{-1} of AA were fed to snakehead (6.6-6.7 g) for eight weeks in the laboratory. Survival, final weight, and specific growth rate (SGR) of snakehead in the 250 and 500 mg.kg^{-1} treatments were significantly greater than those in the control (0 mg.kg^{-1}). Feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) also differed significantly. The requirement of AA for snakehead was calculated to be 277 mg.kg^{-1}. No abnormal backbones were observed during the experimental period. Erythrocyte count was significantly higher at 1000 and 2000 mg.kg^{-1} than at 0 and 125 mg.kg^{-1}. Fish in 250 mg.kg^{-1} had significantly higher leukocyte count than 0, 1000, and 2000 mg.kg^{-1}. A bacterial challenge test with *Aeromonas hydrophila* revealed that 500 and 1000 mg.kg^{-1} had the lowest cumulative mortality. In an on-farm trial, SBM-based and commercial diets augmented with 0, 500, 750, or 1000 mg.kg^{-1} AA were fed to *C. striata* for 23 weeks. Optimal results were obtained with SBM-based diets supplemented with 500 mg.kg^{-1} AA, in terms of survival, yield, FCR, production cost and profit. The result was also confirmed via on-farm trial in Cambodia.

INTRODUCTION

Aquaculture of freshwater carnivorous and omnivorous fish species in Cambodia and Vietnam has been highly dependent on inland fisheries of small-size fish (SSF) for sourcing key dietary nutrient inputs (Hien et al. 2015a). Adequate pelleted diets, with minimal content of fish meal (FM), have been developed to overcome the use of SSF harvested from the Mekong for aquaculture (Hien et al. 2015a; 2016a). In 2015, more than 90% of snakehead farmers (who produce 99% of the total production of snakehead) in 13 provinces in the southern region of Vietnam, including the Mekong Delta, were using these diets instead of SSF, thereby reducing fishing pressure on the small-scale fish in the Lower Mekong Delta (Hien et al. 2016a). However, in a commercial-scale farm trial in An Giang province, about 20% of fish fed this diet (vs. an SSF diet) developed vertebral column abnormalities (Hien et al. 2016b). Anecdotal reports from farmers in the region also indicate that this “hunchback” condition is a problem for them. Pictures and X-rays of the fish suggest that this condition (technically, lordosis and scoliosis) is a classic case of vitamin C (ascorbic acid or AA) deficiency in the diet.

Several benefits have been attributed to AA supplementation in fish such as growth, survival, reduction of skeletal deformities, immunoactivity and stress response (Ai et al. 2006). Dietary AA can enhance resistance to bacterial infection in fish, but AA requirements may depend on species and their physiological conditions (Darias et al. 2011). Fish health is an important issue for snakehead culture; bacterial disease is a serious problem (Duc at al. 2012; 2013). Farmers also indicate that the hunchback problem is greatest when fish are fed to satiation; reduced rations appear to lead to less incidence of the condition. We therefore hypothesize that the condition is due to ascorbic acid (AA) deficiency in the diets of fully fed, fast-growing fish, but that AA levels are sufficient for the slower growth of poorly fed fish. Although we have not found any literature on the specific dietary requirements for AA of our research species, *Channa striata* (or any other member of the family Channidae for that matter), the requirement for most fish species is considered to be 50 $mg.kg^{-1}$ (NRC, 2011). One study of the related *Channa punctata* (Bloch, 1793) indicated that 2000 $mg.kg^{-1}$ of AA provided greater resistance to toxicity of the pesticide endosulfan than did 1000 $mg.kg^{-1}$ (Sarma et al. 2009). Hien et al. (2015a; 2016b) used 80-150 $mg.kg^{-1}$ in laboratory tanks and farm trials, considered to be more than sufficient given the NRC standard cited above, but saw a few abnormal fish in the laboratory experiment and pond trials. In the commercial farm trials, abnormal fish were only seen at the farm in An Giang province, where the fish were grown for 6 months to a size of about 400 g, not at the farm in Dong Thap province, where fish were only grown for 4 months to about 200 g and where farmers added additional vitamin premix to the diet (Hien et al. 2016b). In any case, it appears that additional research on AA requirements for snakehead is necessary to solve this issue of abnormal fish. It may be that the stress on a fast-growing fish like snakehead in a densely stocked pond demands higher levels of AA than were previously anticipated. Cambodia banned snakehead culture in 2005, because the use of SSF by snakehead farmers caused a user conflict with the human population who used SSF as a source of protein. A major rationale for previous research (Hien et al. 2015a,b; 2016a,b; 2017) has been to demonstrate to the Cambodian government that snakehead can be raised profitably on pellet feeds, that snakehead culture methods based on SSF can be discarded, and that the ban could be lifted (which it was in June 2016). The objective of this study was to improve cost-effective feeds for snakehead aquaculture in Vietnam and Cambodia, specifically by determining optimal vitamin C requirement in practical diets in laboratory and pond trials.

OBJECTIVE

To improve cost-effective feeds for snakehead aquaculture in Vietnam and Cambodia, specifically by: (i) determining optimal vitamin C requirement in practical diets in laboratory and pond trials; and (ii) evaluating cost-effectiveness of pellet diets with optimal vitamin C for hapa grow out.

MATERIALS AND METHODS

Laboratory feeding trial

Based on the several benefits attributed to AA in other studies of fish nutrition, and the basal diet from previous work with soybean meal (SBM) replacement of FM in diets for snakehead fish, the null hypothesis that AA level does not lead to differences survival, growth, feed conversion ratio (FCR), protein efficiency ratio (PER), and certain blood parameters of snakehead was tested. A feeding trial was conducted, followed by a bacterial challenge, as outlined below. The lowest supplemented AA value was in the mid-range of what Hien et al. (2015a, 2016a) had previously used, the highest supplemented levels were those used by Sarma et al. (2009), and two intermediate values were also included. The feed was analyzed to verify AA according to methods of Nelis at al. (1997). Treatments were: 1) SBM diet + 0 mg $AA.kg^{-1}$ feed; 2) SBM diet + 125 mg $AA.kg^{-1}$ feed; 3) SBM diet + 250 mg $AA.kg^{-1}$ feed; 4) SBM diet + 500 mg $AA.kg^{-1}$ feed; SBM diet + 1000 mg $AA.kg^{-1}$ feed; SBM diet + 2000 mg $AA.kg^{-1}$ feed (Table 1).

The laboratory experiment was conducted in a manner similar to those of Hien et al. (2015a). Experimental units were 500-L tanks, with five replicate tanks per treatment. The stocking density was 80 fish $tank^{-1}$. At the beginning of the experiment, fish initial weight (6.56-6.77 g) was determined. Fish were fed to satiation twice a day (0800 and 1600 hrs) and the amount of feed consumed by the fish in each tank was recorded daily by removing and weighing (dry weight) excess feed to ascertain intake. Amounts of feed provided per replicate were recorded so that feed conversion ratio (FCR) and protein efficiency ratio (PER) could be calculated at the end of the experiment. Temperature ranged from 27.1 - 30.1 ºC, dissolved oxygen from 5.3 - 5.9 $mg \cdot L^{-1}$, pH from 6.9 – 7.3, TAN from 2.1 – 3.2 and $NO_2^- < 0.1$ $mg \cdot L^{-1}$, so the water quality parameters in all treatments were in a suitable range for the normal growth and development of this species. Any dead fish were recorded and removed daily.

The experiment lasted eight weeks, at the end of which fish were measured, weighed and then used in a bacterial challenge experiment. Blood samples of a subset of experimental fish were taken at the end of the experiment and examined for leukocyte count, erythrocyte count, and lysozyme activity, using methods of Hien et al. (2016b). Any skeletal disorders were documented by photographs and X-rays. Data from each tank were pooled (i.e., no pseudoreplication) and only one number representing average growth per fish (specific growth rate, SGR) was used per replicate. Data analysis was by one-way ANOVA, following arc-sine square-root transformation of the proportionate data to insure normality. Duncan's multiple range test was used to determine specific differences among means if the ANOVA indicated that significant differences were present. Estimation of the vitamin C requirement for fish growth in this trial was by the broken-line regression method (Robbins et al. 1979).

Bacterial challenge experiment

The null hypothesis that AA levels in the feeding trial above do not lead to significantly different survival in a post-trial bacterial challenge was tested. The bacterial challenge experiment was conducted immediately after the end of the feeding trial. The six treatments in the feeding trial were subdivided, such that fish from five tanks per treatment were intraperitoneally (IP) injected with 0.1 mL of bacterial strain *Aeromonas hydrophila* CD1012 based on the lethal dose (LD_{50}) of 1.16×10^5 $CFU.mL^{-1}$ (Duc et al. 2013). Fish from five other randomly chosen tanks were also IP injected with 0.1 mL of physiological saline (0.85%) as control, following Ward et al. (2016). The bacterial challenge experiment lasted 2 weeks, as in the previous work of Hien et al. (2016a). During the 14-d post-inoculation period, fish continued to be fed their respective diets, and activity and cumulative mortality were noted daily. For moribund fish, clinical signs were observed by gross inspection, and lesions were sampled directly for bacteria. Re-isolation and re-identification of bacteria were carried out according to methods of Barrow and Feltham (1993) and PCR was used to speciate the re-identified bacterial strains. Cumulative mortality was recorded daily. Results of the bacterial challenge experiment were analyzed by one-way ANOVA followed by Duncan's multiple range test at significance level of $p \leq 0.05$.

Farm trial in Vietnam

The null hypothesis that source of feed (commercial vs. experimental SBM diet) and levels of added AA do not lead to significant differences in snakehead production to market size in experimental ponds was tested. Based on the results of the laboratory experiment and bacterial challenge, the effects of AA on snakehead in hapas in ponds to simulate farm conditions, was tested using the following treatments: 1) Commercial feed (formulation proprietary, not available); 2) Commercial feed + hand mixed AA at 500 $mg.kg^{-1}$; 3) Commercial feed + hand-mixed AA at 750 mg $AA.kg^{-1}$; 4) Commercial feed + hand mixed AA at 1000 mg $AA.kg^{-1}$; 5) SBM diet (same as Table 1) without AA; 6) SBM diet + 500 mg $AA.kg^{-1}$; 7) SBM diet + 750 mg $AA.kg^{-1}$; 8) SBM diet + 1000 mg $AA.kg^{-1}$. The experiment was conducted in two large experimental ponds at a commercial facility (only SBM diet without AA placed in one pond and the rest in the other pond) with four replicate hapas each. Stocking density was 150 $fish.m^2$ and culture period was 23 weeks until market size was attained.

Data on water quality parameters were collected daily (as described above) and data on fish survival and growth monthly. Production cost (USD.kg $fish^{-1}$) included fingerling cost, vitamin C cost (except control treatment), feed cost, hapa cost and labor cost. Feed cost (USD.kg $fish^{-1}$) was equal to total feed cost (USD) divided by yield (kg). Profit (USD.kg $fish^{-1}$) was equal to selling price (USD.kg $fish^{-1}$) minus production cost (USD.kg $fish^{-1}$). Any skeletal disorders were documented at the end of the experiment by photographs and X-rays. Data on fish survival and growth, FCR, PER and lysozyme were statistically analyzed by two-way analysis of variance.

Farm trial in Cambodia
The grow-out experiment was conducted at Freshwater Aquaculture Research and Development (FARDeC). There were two treatments: 1) Commercial feed; and SBM diet + 500 mg $AA.kg^{-1}$ (same as above), each with 3 replicated hapas allocated in 300 m^2 earthen pond. Local Cambodian snakehead fingerlings (ave.11.2 g size) from the hatchery's F_2 generation, at 60 days old after weaning, were stocked at the density of 450 ind. $hapa^{-1}$ (3x1x1.5 m) to corresponding treatments and replicated hapas for the 5-month grow-out study. Fish in each treatment were fed to satiation twice daily at 09:00h and 16:00h. The amount of feed consumed by fish and fish mortality were recorded daily. Water qualities (temperature, pH, dissolved oxygen, NH_3 and NO_2) were monitored weekly. Fish growth was measured monthly with 30 sampled fish. The survival rate and cannibalism rate were determined at the end of experiment.

RESULTS

Laboratory feeding trial
Survival was significantly higher at 250 and 500 $mg.kg^{-1}$ than in the control (0 $mg.kg^{-1}$), but survival in all treatments with added AA did not differ significantly (Table 2). Final weight and SGR of fish in the 500 $mg.kg^{-1}$ treatments were significantly greater than those in the 125 $mg.kg^{-1}$ treatment, which in turn were significantly greater than those in the control, but there were no differences among the 250, 500, 1000 and 2000 $mg.kg^{-1}$ treatments (Table 2). FI and FCR did not appear to vary in a dose-dependent manner, although some significant differences were seen (Table 2). PER values in all the treatments to which AA had been added were significantly greater than that of the control, but did not differ among themselves (Table 2). Based only on the weight gain data, the vitamin C requirement for the *C. striata* in this experiment was estimated to be approximately 277 $mg.kg^{-1}$ (Figure 1).

Fish in the 1000 and 2000 $mg.kg^{-1}$ treatments had equivalent erythrocyte counts, significantly higher than those in the control and 125 $mg.kg^{-1}$ treatments (Table 3). The highest leukocyte counts were seen in the 125 and 250 $mg.kg^{-1}$ treatments, significantly higher than those in the 1000 and 2000 $mg.kg^{-1}$ treatments, which in turn were significantly higher than those in the control (Table 3). At the end of the feeding trial, lysozyme levels in the control and 2000 $mg.kg^{-1}$ treatments were equivalent, significantly less than those at 250, 500, and 1000 $mg.kg^{-1}$ and the lysozyme levels in the 1000 $mg.kg^{-1}$ treatment were significantly higher than those in any other treatment (Table 3).

No abnormal backbones of snakehead fish were observed during 8 weeks culture in the laboratory trial.

Bacterial challenge experiment
After 14 d of the bacterial challenge, cumulative mortality was significantly greatest, in the control (56%) and 2000 $mg.kg^{-1}$ (54%) treatments, less in the 125 $mg.kg^{-1}$ (30%) and 250 $mg.kg^{-1}$ (34%) and least in the 500 $mg.kg^{-1}$ (20%) and 1000 $mg.kg^{-1}$ (14%) treatments, although the last two listed treatments were not significantly different from each other (Figure 2). Lysozyme levels measured 4 d into the bacterial challenge increased in all treatments compared to those measured prior to the start of the bacterial challenge (Table 3). Lysozyme levels were significantly highest in the 1000 $mg.kg^{-1}$

treatment, followed by those at 500 $mg.kg^{-1}$, which in turn were significantly higher than those at 250 and 150 $mg.kg^{-1}$, which in turn were significantly higher than those at 2000 $mg.kg^{-1}$ and the control (Table 3).

Farm feeding trial in Vietnam

During the 23-week experimental period, all water quality parameters (i.e. temperature, pH, DO, TAN, NO2, and NH3) were similar in the two experimental ponds (Table 5). Results of the two-way ANOVA indicated that diet (commercial vs. SBM) and AA level, as well as their interaction, were significant for all measured variables (final weight, yield, SGR, FI, survival, FCR, lysozyme, hunchback %, production cost, feed cost and profit), with two exceptions. AA level was not significant for hunchback % and diet x AA level interaction was not significant for FCR. More specifically, final weight, yield, survival, FI and lysozyme levels of fish fed SBM with and without AA-supplemented diets were higher than those of the fish fed commercial feed with and without AA-supplemented diets, respectively, and FCR levels were lower (Table 6). The highest values of final weight, yield, and survival were observed in the fish fed with SBM diet + AA 500 $mg.kg^{-1}$, which were significantly different from the control (fish fed SBM diets + 0 $mg.kg^{-1}$ AA); whereas among the treatment groups of commercial diets + AA, these indices were not significantly different (Table 6). FI was highest in the 1000 $mg.kg^{-1}$ AA-supplemented SBM diet group (FI=6.4% $fish^{-1}.day^{-1}$, significantly different from the SBM diet + AA 0 $mg.kg^{-1}$); and the lowest value of FCR, in the 750 $mg.kg^{-1}$ AA-supplemented SBM diet group (FCR=1.27), was significantly different from all commercial diets supplemented with AA ($P<0.05$). Lysozyme levels of the AA-supplemented treatments were higher than those of the control group (no AA supplementation) in both SBM and commercial diets, and the highest lysozyme levels (287.3 and 286.8 $\mu g.mL^{-1}$) were found in fish fed diets containing 1000 $mg.kg^{-1}$ AA-supplemented SBM or commercial feed, respectively.

Fish fed an SBM diet + 500 $mg.kg^{-1}$AA exhibited the lowest percent occurrence of abnormal backbones, production cost, feed cost, and the highest profit (Table 7). The highest percent occurrence of abnormal backbones was found in fish fed commercial diet + 500 $mg.kg^{-1}$ AA. Fish fed the commercial diet + 750 and 1000 $mg.kg^{-1}$ AA exhibited the highest production costs and the lowest profits. The highest percent occurrence of abnormal backbones, in the experimental group of fish fed commercial diet + 750 $mg.kg^{-1}$ AA, was significantly higher than in the control (commercial + 0 $mg.kg^{-1}$ AA).

Farm feeding trial in Cambodia

During the 5-month experiment, growth performance (final weight), daily weight gain and yield of fish in the SBM diet + 500 mg $AA.kg^{-1}$ feed treatment was significantly greater than that in the commercial feed treatment (Figure 3 & 4). Spinal abnormality rate of fish in the SBM diet +500 mg $AA.kg^{-1}$ feed treatment was significantly lower than that in the commercial feed treatment (Figure 5). Survival, cannibalism, and FCR were not significantly different between the two treatments (Table 7). Photographs of the pond trials in Vietnam and Cambodia are provided in Figures 6-10.

DISCUSSION

Our results provide the first estimate of AA requirements in practical diets for *C. striata* grown in ponds in Southeast Asia. Vitamin C is important in multiple processes in the growth, collagen formation, iron metabolism and hematology, reproduction, response to stressors, wound healing and immune response (NRC 2011). Thus, it is not surprising that the diets supplemented with AA promoted better growth of snakehead compared to the control. This result is consistent with the previous studies in hybrid tilapia, *Oreochromis niloticus* (Linnaeus, 1758) × *Oreochromis aureus* (Steindachner, 1864) (Shiau and Hsu, 1995), tilapia, *Oreochromis spilurus* (Günther, 1894) (Al-Amoudi et al. 1992), large yellow croaker, *Pseudosciaena (= Larimichthys) crocea* (Richardson, 1846) (Ai et al. 2006), *O. niloticus* (Ibrahem et al. 2010) and rainbow trout, *Oncorhynchus mykiss*

(Walbaum, 1792) (Adel and Khara, 2016). The finding herein is in agreement with a previous study (Yousefi et al. 2013). Pal and Chakrabarty (2012) and Tewary and Patra (2008), pointed out that supplementation of Vitamin C at 1200 and 1000 $mg.kg^{-1}$ feed yielded better growth of African catfish, *Clarias batrachus* (Linnaeus, 1758) and roho labeo, *Labeo rohita* (Hamilton, 1822), respectively.

Results of experiments on vitamin C may differ due to differences in fish species, size, purity, and sources of ascorbic acid and experimental conditions in different studies. The requirement of vitamin C for snakehead growth in this study was 277 $mg.kg^{-1}$ feed, indicating that the level is higher than that used in previous diet studies with this species (Hien et al. 2015b, 2016b).Vitamin C requirement was 63.37 $mg.kg^{-1}$ feed in *O. niloticus* x *O. aureus* (Shiau and Hsu, 1999), 118 $mg.kg^{-1}$ feed in parrot fish, *Oplegnathus fasciatus* (Temminck & Schlegel, 1844) (Wang et al. 2003) and 43.5 $mg.kg^{-1}$ feed in grouper, *Epinephelus malabaricus* (Bloch & Schneider, 1801) (Lin and Shiau, 2005).

Snakehead in the laboratory experiment fed the SBM diet + 500 mg kg^{-1} AA had the lowest FCR compared to the control, similar to results for African catfish, *Clarias gariepinus* (Burchell, 1822) (Adewolu and Aro, 2009), and significantly greater PER. Vitamin C is known to be positively related to the protein metabolism (Yousefi et al. 2013). Vitamin C is a powerful antioxidant, protecting against oxidative damage to various tissues of fish including red blood cells (Sahoo and Mukherjee, 2003). Red blood cells can act as oxidative status indicators (Pimpimol et al. 2012). In our study, fish fed the diets containing vitamin C had higher total erythrocyte and leukocyte counts than those fed the control diet. Total erythrocyte counts differed significantly between the control and treatments supplemented with AA at 1000 and 2000 $mg.kg^{-1}$. Similar results were observed by Andrade et al. (2007) for pirarucu, *Arapaima gigas* (Schinz, 1822) receiving AA at 800 and 1200 $mg.kg^{-1}$ and by Pimpimol et al. (2012) for Mekong giant catfish, *Pangasianodon gigas* Chevey, 1931 receiving AA at 750 $mg.kg^{-1}$. Moreover, total leukocyte count was significantly increased in snakehead fed AA-supplement diets, which was similar to results in rainbow trout (*O. mykiss*) (Rahimi et al. 2015), tra catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) (Hang et al. 2015) and Japanese eel, *Anguilla japonica* Temminck & Schlegel, 1846 (Shahkar et al. 2015). Leukocytes reached their maximum level at AA-supplementation of 250 $mg.kg^{-1}$ and then declined as AA levels increased beyond that. Thus, AA supplementation may affect the blood composition of snakehead; however, further studies should be considered to evaluate the relationships between vitamin C and other hematological compositions.

Lysozyme activity is an important index of innate immunity of fish and is ubiquitous in its distribution among living organisms (Saurabh and Sahoo, 2008). Lysozyme is liberated by leukocytes and important in anti-microorganism activity (Pimpimol et al. 2012). In our study, lysozyme activity increased in the serum of snakehead fish fed AA-supplemented diets compared to the control, both before and after challenge with *A. hydrophila*. Lysozyme activity increased with level of AA from 250 to 1000 $mg.kg^{-1}$. This is consistent with the previous reports on *P. gigas* fed the dietary AA supplements at $\geq$250 $mg.kg^{-1}$ (Pimpimol et al. 2012) and on improved lysozyme activity with AA supplementation in cobia, *Rachycentron canadum* (Linnaeus, 1766) (Zhou et al. 2012). Our study also revealed that lysozyme activity in snakehead fed diet supplemented with AA at 2000 $mg.kg^{-1}$ was not different from the control. This finding is in agreement with previous studies in channel catfish, *Ictalurus punctatus* (Rafinesque, 1818) (Li et al. 1993), Atlantic salmon, *Salmo salar* Linnaeus, 1758 (Thompson et al. 1993), and *R. canadum* (Zhou et al. 2012) showing lower lysozyme activity at higher AA levels.

The bacterial challenge test indicated that cumulative mortality of snakehead decreased with increased AA levels, at least up to 1000 $mg.kg^{-1}$. Moreover, cumulative mortality was negatively correlated with the total erythrocyte and leukocyte counts and lysozyme activity. Similar results were found in *R. canadum* (Zhou et al. 2012). This finding demonstrates the relationships among dietary AA supplementation, immunological parameters, and disease resistance. However, our highest AA-supplemented diet (2000 $mg.kg^{-1}$) did not appear to be effective for disease resistance, which has been demonstrated previously by Li et al. (1993).

Results of the farm feeding trial were consistent with those of the laboratory feeding trial, demonstrating that dietary vitamin C can significantly improve the normal growth and other physiological functions in snakehead. Fish fed diets with AA supplemented at 500 mg.kg-1 in the farm trial (i.e., the next highest treatment level above the AA requirement calculated from growth results in the laboratory trial) yielded the lowest feed costs and highest profits. Additionally, the farm feeding trial showed that the SBM diet was a viable feed option for use in aquaculture of snakehead compared with the commercial feed. It appears that the diet X AA level interaction in the farm trial was largely due to AA level having much less effect on the commercial diet than on the SBM diet. We speculate that this was caused by hand-mixing of the AA into the commercial diet, rather than fully incorporating it into the diet during production.

CONCLUSIONS

In conclusion, this study demonstrated that dietary AA supplementation is able to improve growth performance, immune responses, and survival of snakehead fish against *A. hydrophila* infection. The requirement of AA by snakehead was determined as 277 $mg.kg^{-1}$ feed in the laboratory study. The diet supplemented with vitamin C at 250 to 1000 $mg.kg^{-1}$ feed is an appropriate concentration range for improving growth performance and immunity of snakehead fish on fish farms. SBM diet was a better diet used in culturing snakehead compared with the commercial diet. Further experiments should be considered to investigate correlations between vitamin C and other immune parameters, as well as disease resistance of snakehead against other pathogens.

QUANTIFIABLE ANTICIPATED BENEFITS

In Vietnam:

1. Development of weaning strategies for snakehead;
2. Development of pellet diets for snakehead in Vietnam;
3. Investigation of vitamin C requirements of snakehead in Vietnam;
4. Transfer of snakehead culture technology to Cambodia;
5. Training snakehead culture to women in An Giang, Dong Thap and Tra Vinh provinces;
6. Two graduate and two under-graduate student were done their thesis research in this investigation.

In Cambodia:

This research provides information on growing out of snakehead fish using vitamin C supplemented feed to for improving growth performance and production on snakehead farm in Cambodia. The following are quantifiable anticipated benefits:

- Scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staffs, private sector and university lecturers and students working on the issues of snakehead aquaculture in Cambodia as well as in other Mekong riparian countries were better informed about research methods and findings,and have better recommended policies and strategies for sustainable snakehead aquaculture.
- One undergraduate student was supported and trained by this investigation through B.Sc. thesis research.

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TABLES AND FIGURES

Table 1. Ingredients used in, and proximate composition of, diets used in the snakehead laboratory feeding trial with varying levels of L-Ascorbate-2-Monophosphate.

Ingredients	**Composition (%)**
Kien Giang fishmeal	35.8
Defatted soybean meal	33.4
Cassava	8.26
Rice bran	15.0
Premix mineral and vitamins	2.0
Oil	3.08
Carboxymethyl cellulose	0.40
Lysine	0.40
Methionine	0.28
Fish solution	1.50
Phytase	0.02
Total	**100**
Crude protein	45.0
Crude lipid	8.91
Ash	12.5
Fiber	2.15
NFE	31.5
Energy ($kJ.g^{-1}$)	19.69

Premix mineral and vitamin ($unit.kg^{-1}$): Vitamin A 2,000,000 IU; Vitamin D 400,000 IU; Vitamin E 6g; Vitamin B_1 800mg; Vitamin B_2 800mg; Vitamin B_{12} 2mg; Calcium D Pantothenate 2g; Folic acid 160mg; Choline Chloride 100g; Iron (Fe^{2+}) 1g; Zinc (Zn^{2+}) 3g; Manganese (Mn^{2+}) 2g; Copper (Cu^{2+}) 100mg; Iodine (I^-) 20mg; Cobalt (Co^{2+}) 10mg. Fishmeal was from Kien Giang. Cassava and rice bran were local products. CMC; methionine and lysine were products of Evonik.

Table 2. Initial and final weights.fish^{-1}, specific growth rate, SGR), feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) of *Channa striata* fed diets with different levels of vitamin C (as L-ascorbate-2-monophosphate) for 8 weeks. Values (mean±SD in parentheses) in a column sharing a superscript letter are not significantly different (*P>0.05*).

Target vitamin C supplementation (mg kg-1)	Initial weight (g)	Final weight (g)	SGR (%.d^{-1})	Survival rate (%)	FI (%.fish^{-1}.day^{-1})	FCR	PER (%)
0	6.57^{a}	39.06^{a}	2.97^{a}	76.5^{a}	3.99^{ab}	1.18^{c}	1.52^{a}
	(0.09)	(2.63)	(0.13)	(6.3)	(0.27)	(0.04)	(0.14)
125	6.56^{a}	45.45^{b}	3.22^{b}	84.8^{ab}	4.27^{abc}	1.14^{bc}	1.79^{b}
	(0.18)	(3.66)	(0.12)	(3.5)	(0.07)	(0.07)	(0.12)
250	6.61^{a}	48.07^{bc}	3.31^{bc}	88.8^{b}	4.24^{abc}	1.09^{abc}	1.8^{b}
	(0.18)	(1.93)	(0.09)	(7.7)	(0.21)	(0.04)	(0.15)
500	6.60^{a}	51.8^{c}	3.43^{c}	86.3^{b}	4.29^{bc}	1.06^{ab}	1.92^{b}
	(0.10)	(4.17)	(0.14)	(6.7)	(0.16)	(0.09)	(0.11)
1,000	6.68^{a}	49.1^{bc}	3.32^{bc}	83.5^{ab}	3.97^{a}	1.02^{a}	1.9^{b}
	(0.26)	(4.34)	(0.14)	(6.4)	(0.24)	(0.12)	(0.16)

Table 3. Effect of Vitamin C (as L-ascorbate-2-monophosphate) supplemented feed on hematology and immune response of *Channa striata.* Pre-challenge lysozyme levels are after 8 weeks of the laboratory feeding trial, whereas post-challenge lysozyme levels are after 8 weeks of the laboratory feeding trial plus four days of the bacterial challenge. Values (mean±SD in parentheses) in a column followed by the same superscript are not significantlhy different.

Target Vitamin C supplementation (mg kg-1)	Total erythrocytes count (x10^{5}cells.mm^{3})	Total leukocytes count (x10^{3}cells.mm^{3})	Pre-challenge[1] lysozyme (µg.mL^{-1})	Post-challenge[2] Lysozyme (µg.mL^{-1})
0	22.8^{a}	108.1^{a}	205.0^{a}	241.1^{a}
	(8.9)	(51.3)	(42.5)	(29.3)
125	31.5^{a}	355.8^{c}	214.5^{ab}	328.2^{b}
	(6.6)	(80.5)	(30.7)	(25.7)
250	32.4^{ab}	385.1^{c}	274.5^{bc}	351.5^{b}
	(5.2)	(53.7)	(31.8)	(17.6)
500	35.1^{ab}	313.2^{bc}	313.0^{c}	413.2^{c}
	(6.7)	(66.2)	(46.6)	(62.5)
1,000	44.7^{b}	263.1^{b}	372.5^{d}	506.5^{d}
	(6.8)	(58.8)	(24.3)	(46.7)
2,000	45.1^{b}	230.8^{b}	212.0^{a}	264.0^{a}
	(16.5)	(83.1)	(41.1)	(15.6)

Table 4. Water quality parameters in two experimental ponds (control and treatment, see text) used in the Vietnam farm trial with *C. striata* during the 23 weeks of culture. Values are mean±SD (in parentheses).

Experimental pond	Temperature (°C)		pH		DO ($mg.L^{-1}$)		TAN ($mg.L^{-1}$)	NO_2 ($mg.L^{-1}$)	NH_3 ($mg.L^{-1}$)
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon			
Control	29.3	31.5	8.2	8.3	1.6	3.8	3.48	0.004	0.030
	(0.90)	(0.63)	(0.1)	(0.1)	(0.4)	(0.2)	(0.35)	(0.002)	(0.010)
Treatment	29.2	31.3	8.1	8.2	1.8	3.7	0.55	0.061	0.005
	(0.94)	(0.86)	(0.1)	(0.2)	(0.3)	(0.4)	(0.14)	(0.016)	(0.001)

Table 5. Initial and final weights.fish-1, yield, survival, feed intake (FI), feed conversion ratio (FCR), and lysozyme of *Channa striata* fed soybean meal (SBM) diet and commercial feed (CF) with different levels of Vitamin C (as L-ascorbate-2-monophosphate for 23 weeks in the Vietnam farm trial. Values (mean±SD in parentheses) in a column sharing a superscript letter are not significantly different (*P>0.05*). Results of the two-way ANOVA are given at the bottom.

Diet and target vitamin C supplementation (mg kg-1)	Initial weight (g)	Final weight (g)	Yield (kg/hapa-4m^2)	Survival (%)	FI (%.fish^{-1}.day^{-1})	FCR	Lysozyme (µg.mL^{-1})
SBM-0	9.00	461.7^a	190.6^a	69.1^a	5.40^a	1.40^{bc}	232.9^a
	-	(40.1)	(8.9)	(5.8)	(0.40)	(0.11)	(4.8)
SBM-500	9.00	573.5^c	293.3^c	85.3^c	5.69^{ab}	1.16^a	271.9^b
	-	(34.3)	(10.8)	(3.2)	(0.25)	(0.06)	(17.6)
SBM-750	9.00	556.2^c	268.3^{bc}	80.4^{bc}	6.00^{abc}	1.27^{ab}	283.7^b
	-	(19.2)	(8.6)	(2.2)	(0.05)	(0.01)	(4.9)
SBM-1000	9.00	565.4^c	264.6^b	78.3^{bc}	6.40^c	1.35^{bc}	287.3^b
	-	(44.2)	(7.5)	(4.7)	(0.55)	(0.12)	(9.5)
CF-0	9.00	398.9^{ab}	172.1^a	71.9^{ab}	6.07^{abc}	1.50^c	228.6^a
	-	(41.2)	(18.2)	(3.0)	(0.11)	(0.07)	(10.6)
CF-500	9.00	399.8^{ab}	185.9^a	77.7^{abc}	6.03^{abc}	1.47^c	224.1^a
	-	(28.1)	(9.1)	(4.2)	(0.19)	(0.08)	(16.8)
CF-750	9.00	398.0^{ab}	177.7^a	74.6^{ab}	6.08^{bc}	1.49^c	227.9^a
	-	(38.9)	(13.3)	(3.8)	(0.10)	(0.08)	(13.7)
CF-1000	9.00	376.0^a	173.3^a	76.8^{abc}	6.08^{bc}	1.48^c	286.8^b
	-	(22.6)	(12.0)	(1.8)	(0.05)	(0.02)	(22.9)
P values							
Feed		0.000	0.000	0.033	0.050	0.000	0.000
Vitamin C		0.018	0.000	0.000	0.013	0.017	0.000
*Feed*Vitamin C*		0.006	0.000	0.049	0.020	0.051	0.001

Table 6. Hunchback (abnormal backbones), production costs (PC), feed cost, and profit (P) for fish raised on soybean meal (SBM) diet and commercial feed (CF) with different levels of Vitamin C (as L-ascorbate-2-monophosphate for 23 weeks in the Vietnam farm trial. Values (mean±SD in parentheses) in a column sharing a superscript letter are not significantly different (*P>0.05*)

Diet and target vitamin C supplementation (mg kg-1)	Hunchback fish (as % of total)	Production cost (USD.kg fish^{-1})	Feed cost (USD.kg fish^{-1})	Profit (USD.kg fish^{-1})
SBM-0	6.28^{ab}	1.39^{bc}	1.18^{b}	0.12^{ab}
	(0.68)	(0.10)	(0.09)	(0.10)
SBM-500	5.37^{a}	1.12^{a}	0.98^{a}	0.38^{c}
	(0.85)	(0.06)	(0.05)	(0.06)
SBM-750	5.89^{a}	1.23^{ab}	1.07^{ab}	0.27^{bc}
	(0.61)	(0.02)	(0.01)	(0.02)
SBM-1000	6.75^{abc}	1.31^{bc}	1.14^{b}	0.20^{ab}
	(1.08)	(0.10)	(0.10)	(0.10)
CF-0	8.32^{bcd}	1.43^{c}	1.19^{b}	0.08^{a}
	(0.98)	(0.08)	(0.05)	(0.05)
CF-500	9.63^{de}	1.40^{bc}	1.17^{b}	0.11^{a}
	(1.49)	(0.07)	(0.06)	(0.07)
CF-750	11.1^{e}	1.43^{c}	1.19^{b}	0.07^{a}
	(0.81)	(0.06)	(0.06)	(0.05)
CF-1000	8.91^{cde}	1.44^{c}	1.18^{b}	0.07^{a}
	(1.23)	(0.04)	(0.02)	(0.04)
P values				
Feed	0.000	0.000	0.000	0.000
Vitamin C	0.118	0.003	0.017	0.003
*Feed*Vitamin C*	0.009	0.025	0.049	0.015

Table 7. Survival rate (SR), hunchback (abnormal backbones), weight gain (Wg), feed conversion rate (FCR) and yield of *Channa striata* of Cambodian origin during grow-out. Values (mean±SD in parentheses) in a column followed by the same letter are not significantly different (P>0.05)

Treatments	SR (%)	Hunchback (%)	Wg ($g.fish^{-1}$)	FCR	Yield (kg/hapa)
SBM+500mg $AA.kg^{-1}$ Feed	45.7[a] (1.4)	21.7[a] (1.6)	322.8[a] (11.2)	1.9[a] (0.1)	65.0[a] (3.2)
Commercial Feed	45.7[a] (13.7)	35.0[a] (14.6)	203.8[b] (10.7)	2.8[b] (0.5)	40.3[b] (10.7)

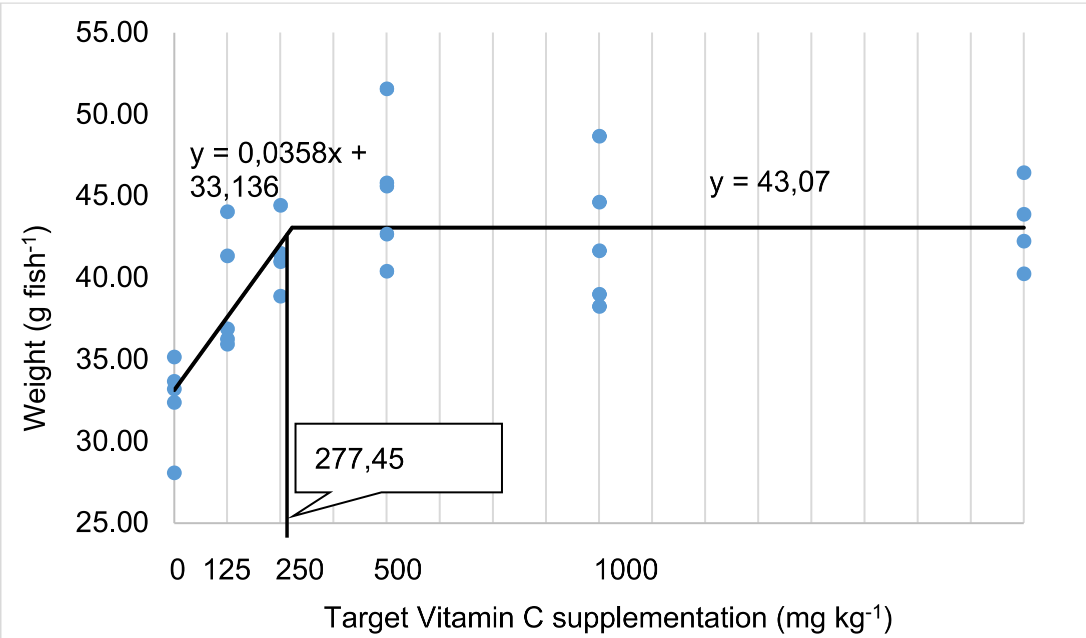

Figure 1. Requirement of dietary vitamin C on growth responses of snakehead fish, C. striata.

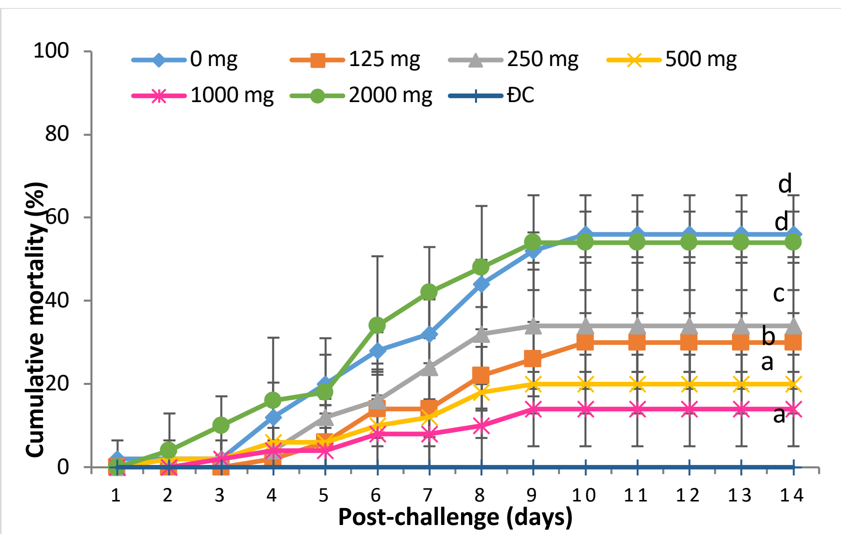

Figure 2. Cumulative mortality over 14 d in snakehead fish, *Channa striata*, fed diets with different levels of Vitamin C (as L-ascorbate-2-monophosphate) for 8 weeks, then inoculated intraperitoneally with *Aeromonas hydrophila* in the bacterial challenge experiment.

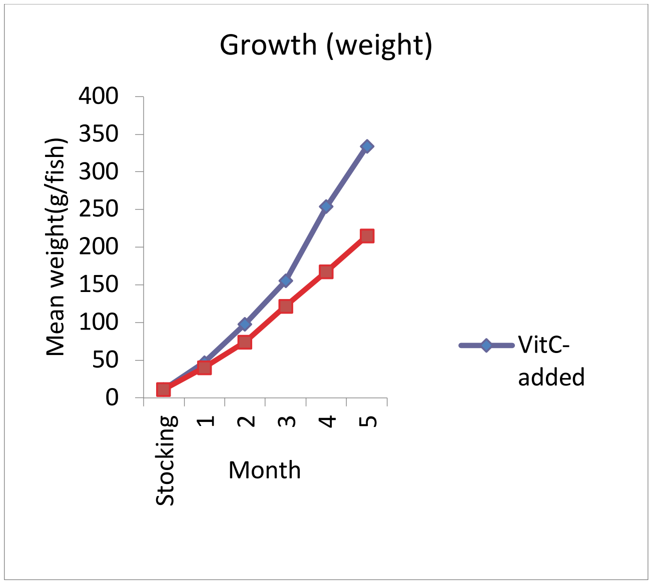

Figure 3. Growth performance of Cambodian snakehead *Channa striata* during 5-month grow-out

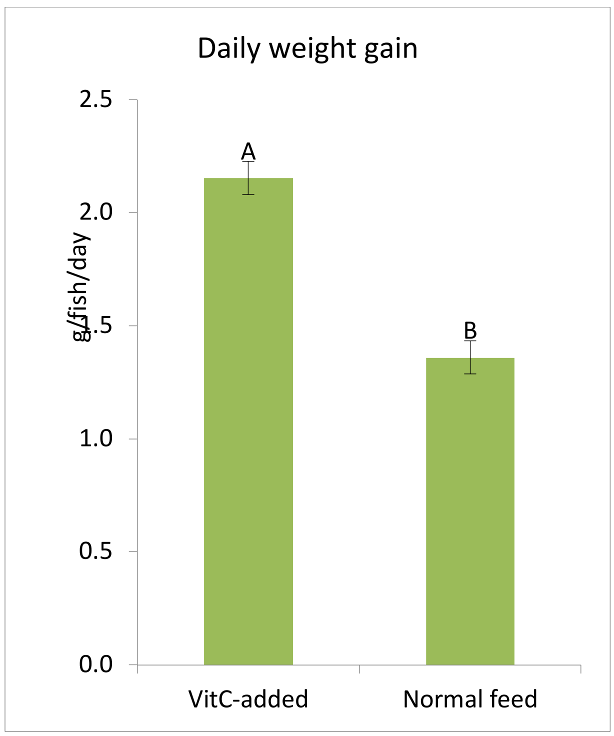

Figure 4. Daily weight gain (g $fish^{-1}$ day^{-1}) of Cambodian snakehead *Channa striata* during 5-month grow-out

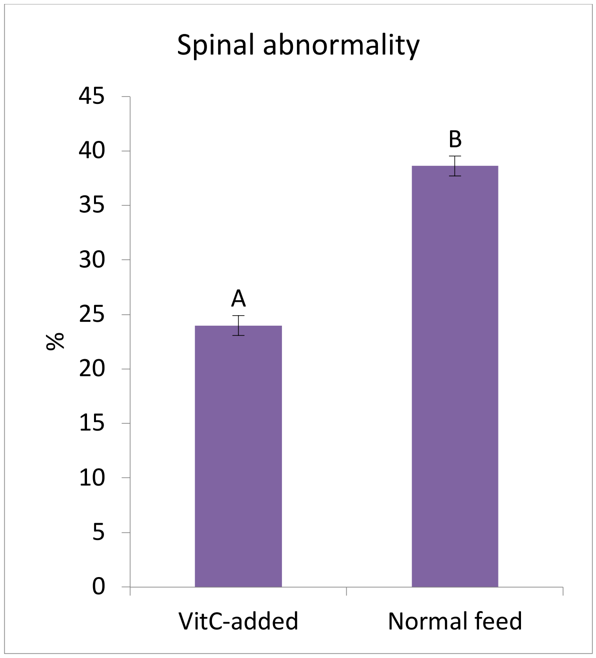

Figure 5. Spinal abnormality rate (%) of Cambodian snakehead *Channa striata* during 5-month grow-out

Figure 6. Grow-out hapas in Vietnam

Figure 7. Harvesting snakehead from grow-out hapas in Vietnam

Figure 8. Grow-out hapas (3x1x1.5m) in Cambodia

Figure 9. Cambodian snakehead fingerling (60 day-old) at stocking

Figure 10. SBM diet (including 500 $mgAA.kg^{-1}$ feed) for hapa study in Cambodia.

NUTRITIONAL CONDITIONING DURING LARVAL DEVELOPMENT TO IMPROVE PRODUCTION AND FEED EFFICIENCY AND ESTABLISHMENT OF BENEFICIAL GUT FLORA IN TILAPIA CULTURE

Sustainable Feed Technology and Nutrient Input Systems/Experiment/16SFT02NC

Scott A. Salger[1], Kaniz Fatema[2], Courtney A. Deck[1], Nusrat Hossain Nushy[2], Md Mehedi Hasan[2], Jakir Hossain[2], , Hannah M. Reynolds[1], Peter R. Ferket[3], Md Abdul Wahab[2], S. Mahean Haque[2], and Russell J. Borski[1]

[1]*Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA*
[2]*Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, Bangladesh*
[3]*Department of Poultry Sciences, North Carolina State University, Raleigh, NC, USA*

ABSTRACT

Feed constitutes 60-80% of total production costs of tilapia (*Oreochromis spp.*) with high quality protein being the most expensive component. Studies in mammals have demonstrated that early nutrient contributions can influence growth and other key physiological processes. This phenomenon, known as nutritional conditioning, was also shown in chickens and fish where limiting a nutrient in the early stages of development led to enhanced uptake and utilization later in life. We evaluated if this process could be applied to tilapia culture in the form of protein-restriction as this could reduce the cost of feed and thus production of tilapia for small-scale rural farmers in Bangladesh. It has yet to be determined in fish if protein restriction early in life might lead to improved growth later on. We first performed a tank trial to determine the effectiveness of protein-restriction in tilapia culture. Post-yolk sac fry were fed a 25% crude protein (CP) starter diet for 7, 14, or 21 days or a typical 48% CP diet for 21 days after which half the tanks from each treatment were fed a reduced 25% CP growout diet while the other half received a typical 38% CP growout diet. The results indicate that fish fed a reduced protein diet initially had significantly greater lengths and weights at the end of the 56-day study period relative to controls, however growth and survival decreased when fish remained on the 25% CP starter diet for longer than 14 days. Our RNA-seq analysis of tilapia intestinal tissue showed differential expression of genes involved in proteolysis and muscle function between groups receiving different levels of protein which may indicate compensatory mechanisms for maintaining function during protein restriction or enhancing muscle synthesis when dietary protein levels are increased. Additionally, tilapia fed an initial protein-restricted diet for 14 days had the greatest microbe diversity in their fecal matter. A more diverse microbiome is associated with enhanced health and feed efficiency and thus the use of nutritional conditioning in tilapia culture may be beneficial for more than simply increasing the growth of the fish. We also assessed whether this nutritional conditioning would also be effective for pond culture in Bangladesh. Here, post-yolk sac fry were fed a reduced 20% CP starter diet or a typical 40% starter diet for 15 days and then switched to a 20% CP or 40% CP growout diet through 90 days. Contrary to the tank study, we determined that fish fed a reduced protein diet initially had lower growth and production yields than those fed the 40% CP diet. It is likely that fish on the protein restricted diet had access to natural food sources within ponds and hence were not truly restricted enough to yield a nutritional programming response as was seen in tank trials. Early restriction of protein under more controlled conditions in hatcheries followed by growout in ponds should be tested. Interestingly, the fish that were switched from the 40% CP started diet to the 20% CP growout diet had higher growth and survival as well as the greatest net returns. Thus, while a nutritional conditioning response was not observed, it may still be possible to lower feed costs by reducing the amount of protein provided during the growout stage. These are the first studies to demonstrate that nutritional programming or protein restriction early in life can improve growth of fingerling tilapia under controlled conditions. Future work should evaluate whether improved feed efficiency and growth can be sustained throughout growout in fish subjected to early protein restriction.

INTRODUCTION

Global production of farmed Nile tilapia (*Oreochromis niloticus*) has increased exponentially since 1985, with over 2.4 million metric tons consumed in 2010 (FAO, 2013). In Bangladesh, Nile tilapia comprises a significant source of per capita caloric and protein intake, with production increasing 30-fold from 1999-2007 (Hussain, 2009). Total production is second only to carps (Apu, 2014). Feed ingredients typically include fishmeal, other animal meal or byproducts, and plant material (soybean products) as primary sources of protein for fish growth. High quality protein is critical for animal growth and health and requirements vary depending on age and size of the fish. Commercial feeds for pond growout contain ~30% protein while juvenile tilapia may require up to 40% protein for proper growth (El-Sayed, 2006). Work in mammalian models demonstrates that nutrient contributions early in pre- or postnatal development influence growth and immune function later in life (Lucas, 1998). This process, known as conditioning (also programming or imprinting), when established at critical periods in the animal's development, lead to life-long changes in the function of key elements of an organism's physiology. By altering early nutritional components, nutritional conditioning can result in more efficient uptake and utilization of nutrients from the diet thus increasing growth and health parameters in the organism later in life. The process of nutritional conditioning is also observed in poultry. Nutritional conditioning of energy and minerals can influence uptake and utilization in chickens (Ashwell and Angel, 2010; Ferket, 2013). Broiler chickens had increased retention of phosphorous from their diets following feeding of a phosphorous-deficient diet for the first 90 hours post-hatch (Ashwell and Angel, 2010).

A better understanding of how finfish acquire and utilize nutrient inputs is requisite for future improvements in aquaculture production efficiency as feed constitutes anywhere from 50% to 80% of total variable production costs. Nothing is known about the effectiveness of applying nutritional conditioning to tilapia culture, this despite strong evidence that the phenomenon is likely to occur across all vertebrate taxa. A few studies have looked at energy uptake and utilization following conditioning in rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*), both carnivorous fishes. High dietary glucose diets fed to rainbow trout juveniles for a short period showed there was long-term modifications to carbohydrate metabolism (Geurden et al., 2007) while European sea bass juveniles fed a HUFA-deficient diet initially were able to metabolize lipids more efficiently than those fed a high HUFA diet (Vagner et al., 2007). Here we evaluated if nutritional conditioning can be applied to tilapia and assessed whether reductions in the amount of protein in the diet of post-larval tilapia would subsequently lead to improved feed or protein efficiency during later growout of fishes.

Currently, the underlying mechanisms explaining how larval nutritional conditioning strategies can potentially achieve equivalent production yields with less protein in the feed is poorly understood. Some evidence suggests that during periods of fasting, nutrient uptake efficiency in the intestine is intrinsically enhanced, leading to a more-efficient uptake of nutrients at the next feeding period (Ali et al., 2003; Picha et al., 2006). Thus, decreasing the amount of select nutrients early in the life of the fish may increase the uptake and utilization of those nutrients during the growout phase of fish culture. Using a transcriptomic approach, we evaluated the suite of genes in the intestine that are differentially expressed in fish fed a protein deficient diet early in larval development versus those receiving a diet containing traditional levels of protein to further our understanding of how protein assimilation and enhanced growth may be achieved for greater optimization of feeding protocols in the future.

Nutritional conditioning may also affect the microbial colonization of the gut. The establishment of beneficial microflora can affect nutrient availability and gut health (Marques et al., 2010). The emerging field of metagenomics has substantial implications for sustainable aquaculture, as diet, feeding strategy, and other environmental factors strongly influence the diversity and constitutive abundance of intestinal microbiota in both humans and fish (Al-Harbi and Uddin, 2004, 2005; De Filippo et al., 2010; Heikkinen et al., 2006). In cultured finfish, new research has shown that probiotic maintenance of beneficial gut flora can promote growth, enhance nutrient availability, and increase overall stock health (Nayak, 2010; Welker and Lim, 2011). In our previous AquaFish Innovation Lab studies, we found that tilapia fed on alternate days in fertilized ponds produced similar growth and survival, but improved feed efficiency by 100%, compared with fish fed daily. Fish also had a higher diversity of microbes in their intestines that may benefit nutrient processing and uptake. Here we built on this work to determine whether tilapia intestinal microbial composition and diversity varies with nutritional conditioning and identify key microbes that may be associated with increased protein uptake and utilization. The identification of such microbes may benefit current research into the application of probiotic supplements in fish culture for further enhancement of feed efficiency.

This investigation targeted a method to improve production efficiency of tilapia, namely through reducing the amount and cost of feed needed to produce a kg of fish. Since >50% of the costs associated with feed is protein, practical approaches that improve its utilization has tremendous application to global tilapia production (El-Sayed, 2006). Previously, our research showed that Nile tilapia and milkfish (seacages and ponds; *Chanos chanos*) can be grown to market size in monoculture with significant cost savings through implementation of alternate-day feeding versus daily feeding (50% feed reduction; Bolivar et al., 2006; Borski et al., 2011; De Jesus-Ayson and Borski, 2012). Our work over the past year also indicates similar responses with tilapia grown in ponds in Bangladesh. Here we determined the proper length of time for nutritional conditioning of post-yolk sac Nile tilapia fry that can lead to enhanced feed efficiency with minimal impact on growth and survival of the fish in a laboratory setting and subsequently investigated whether this strategy could be applied to pond culture in Bangladesh where it would have the potential to provide substantial costs savings for tilapia farmers and also mitigate negative environmental impacts associated with excessive nutrient loading.

OBJECTIVES

1. Evaluate the effectiveness of nutritional conditioning on tilapia growth.
2. Identify key factors (gene networks) associated with improved growth in response to larval nutritional conditioning in tilapia.
3. Characterize changes in gut microbial communities in response to nutritional conditioning and identify those that may be associated with improved nutrient absorption and growth in fish.

MATERIALS AND METHODS

Study 1 - Evaluate the effectiveness of nutritional conditioning on tilapia growth

The initial laboratory investigations were performed at North Carolina State University. Restricted and normal protein diets were formulated by Integral Fish Foods Inc. (Albany, Indiana) using the nutritional profiles outlined by Mjoun et al. (2010) as a guideline (Table 1) and feed was produced by the Bozeman Fish Technology Centre (Bozeman, Montana). Post-yolk sac Nile tilapia fry were obtained from the Louisiana Speciality Aquafarm (Harvey, LA) and stocked in 36 experimental tanks (2.8 L) at the Grinnell's Fish Laboratory (NCSU). The study consisted of 8 treatment groups in which fry were fed a normal (48% CP) or restricted (25% CP) protein starter diet for 7, 14, or 21 days before being switched to a normal (38% CP) or restricted (25% CP) protein growout diet for the remainder of the 56-day study period (Figure 1). Each

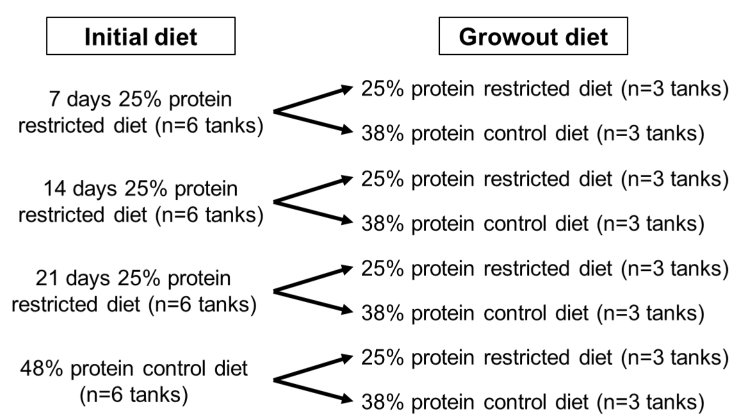

Figure 1. Experimental design for tank study.

treatment was replicated in 3 separate tanks. Fish were fed 3 times a day to satiation for the first 28 days after which this was reduced to twice a day to satiation and tanks were cleaned regularly to remove algae and feces that the tilapia could consume. Growth parameters (length and weight) were measured throughout the study period (sub-sampling) and survival rates for each treatment were calculated at the end of the experiment. Intestinal samples for RNA-Seq analysis were taken from a subset of fish at the end of 56 days and placed in RNA Later (Invitrogen). In the microbiome analyses tilapia fry up to 15 mm TL were too small to dissect out individual intestinal tissues for sampling. Instead, whole fry were rinsed in 70% ethanol and sterile deionized water to wash off external microbes that may be found on the skin of the fish. In animals > 15 mm, fecal material was collected from the posterior intestine (colon) and placed in a vial containing buffer from a Quick-DNA Fecal/Soil Microbe Kit (Zymo Research) and bullet homogenized with a portable homogenizer. Samples collected from fish were pooled together according to treatment group (samples from 2 fish per tank pooled, 2 pooled samples per tank, 3 tanks per treatment; N = 6 per treatment). The pooled sample design was used to offset potential variability of microbiota within individuals, instead focusing on common patterns, which may be more reflective of changes with treatment group among the population as a whole. Samples were collected at 7, 14, 21, 28, 35, and 56 days after start of the study.

Table 1. Nutrient composition of diets used in the tank study. ME = metabolizable energy.

	25% Starter	48% Starter	25% Growout	38% Growout
Protein	25.00%	48.00%	25.00%	38.00%
Fat	12.13%	8.58%	9.59%	8.00%
Carbohydrates	36.72%	15.88%	36.59%	23.80%
Fibre	3.64%	2.04%	4.78%	3.96%
Ash	4.47%	6.38%	5.17%	6.15%
Lysine	1.96%	3.80%	1.45%	2.19%
Methionine	0.45%	1.08%	0.44%	0.66%
Tryptophan	0.30%	0.50%	0.31%	0.48%
ME (Catfish)	2584 kcal/kg	1986 kcal/kg	2353 kcal/kg	2350 kcal/kg
Fishmeal	10.08%	24.67%	9.85%	8.47%
Poultry products	0%	19.74%	0%	14.05%
Wheat grain	25.19%	14.80%	14.77%	15.06%
Soybean seeds	1.68%	9.87%	9.85%	25.10%
Wheat flour	33.59%	9.87%	49.24%	23.42%

While the use of tanks for aquaculture is common practice in the US, developing countries such as Bangladesh primarily culture their fish in ponds. Thus, we evaluated whether restricted protein diets could be applied to pond cultures to help reduce feed costs for poor rural farmers. These studies were performed at the Fisheries Field Laboratory, Bangladesh Agricultural University. Normal and restricted protein feeds were produced by Krishibid Feed Ltd. (Mymensingh, Bangladesh) (Tables 2, 3). A total of 7500 Nile tilapia post-yolk sac fry were obtained from Reliance Aqua Farm (Mymensingh, Bangladesh) and stocked in 12 hapas at the field laboratory. Fry in 6 hapas received a 20% CP diet and the other 6

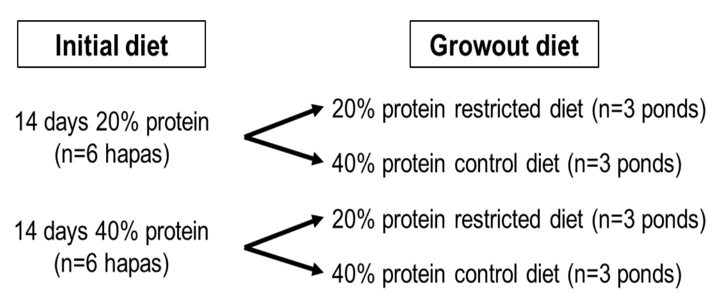

Figure 2. Experimental design for pond study.

received a 40% CP diet for 14 days at a rate of 15% BW per day, after which the fish from each hapa were transferred into one of 12 rectangular ponds, averaging 130 m^2 and 1.5 m deep. Ponds had previously been limed with $CaCO_3$ (1 kg/dec) and were fertilized with urea (28 kg N/ha) and triple super phosphate (7 kg P/ha) 7 days prior to fish stocking. Once in the ponds, tilapia were fed either a 20% or 40% CP growout diet as illustrated in Figure 2. Feed was provided at a rate of 15% BW initially before being reduced to 8% BW. Additionally, all ponds were fertilized weekly with urea and TSP. Water temperature, transparency, pH, dissolved oxygen, nitrates, nitrites, phosphates, total dissolved solids, alkalinity, conductivity, and chlorophyll-a were assessed weekly. Phytoplankton and zooplankton abundance were also measured. Data were analysed by one-way ANOVA followed by a Tukey's multiple comparison test.

Table 2. Composition of the starter feeds for the pond study.

	20% Crude Protein	40% Crude Protein
Fish meal	5.00%	25.00%
Meat and bone meal	9.00%	21.00%
Mustard oil cake	15.00%	15.00%
Rice bran	50.00%	18.25%
Chemical binder	5.00%	5.00%
Soybean meal	0.00%	15.00%
Maize	25.25%	0.00%
Salt	0.50%	0.50%
Vitamin premix	0.25%	0.25%

Table 3. Composition of the growout feeds for the pond study.

	20% Crude Protein	40% Crude Protein
Wheat flour	15.00%	19.00%
Corn products	22.80%	3.00%
De-oiled rice bran	30.00%	0.00%
Soybean meal	0.00%	30.85%
Full fat soybean	3.55%	10.00%
Rape seed meal	20.00%	20.00%
Meat and bone meal	0.00%	10.00%
Poultry oil	2.26%	1.70%
Methionine	0.11%	0.40%
Lysine sulphate	0.46%	0.79%
Fish vitamin	0.25%	0.25%
Salt	0.36%	0.25%

Study 2 – Identify key factors (gene networks) associated with enhanced nutrient utilization in response to larval nutritional conditioning in tilapia

Total RNA from tilapia anterior intestinal samples from Study 1 were extracted using TRI Reagent (Molecular Research Centre, Inc.) and sent to the NCSU Genomic Sciences Laboratory for library preparation and RNA-Seq analysis using the Illumina HiSeq platform. For each treatment, a bar-coded amplicon library (n=2-4) was constructed and the pooled sample was run on a single Illumina lane (125 bp, single end reads). The NCSU Bioinformatics Consulting and Service Core (BCSC) was then used for the High Performance Computing environment with the CLC Workbench license to identify differentially expressed genes (DEG) between each treatment group and the control (48% protein to 38% protein). The residuals of each highly significant DEG were put into a Modulated Modularity Clustering program (Stone and Ayroles, 2009) to group the highly significant differentially expressed genes while Gene Ontology (GO) term enrichment of each module determined cluster function using DAVID Bioinformatics suite (Huang et al., 2009) and Zebrafish Information Network (ZFIN) GO annotation.

Study 3 – Identification of key microbial factors promoting increased nutrient absorption by enterocytes in the tilapia gastrointestinal tract

Ribosomal RNA extraction

Extraction of ribosomal RNA from whole tilapia (9 mm to 15 mm TL) or tilapia posterior intestinal fecal samples (greater than 15 mm TL) was performed using a Quick-DNA Soil/Fecal DNA Microbial Kit (Zymo Research, Corp., Irvine, CA) following the included protocol. Up to 0.25 g of fecal sample was placed into a ZR BashingBead Lysis Tube with 750 µL Lysis/Stabilization Solution. The tube was secured in an Xpedition Sample Processor and processed for 30 s and stored at room temperature until extraction. The concentration and quality were determined by Nanodrop (Thermo Fisher Scientific, Inc., Waltham, MA). The extracted rRNA was stored at -20°C for sequencing library preparations.

Prokaryotic 16S rRNA sequencing library preparation

Prokaryotic 16S rRNA gene amplicons were prepared following the 16S Metagenomic Sequencing Library Preparation protocol for the Illumina MiSeq system with some modifications. Primers were designed to amplify the V3 to V5 regions of 16S rRNA (Muyzer et al. 1993; Sim et al. 2012) with overhang adapter sequences compatible with the Illumina index and sequencing adapters and allowed for double indexing to increase the accuracy of the multiplexed reads. Amplicon PCR was used to amplify the region of interest from the gDNA extracted from the tilapia fecal material samples. The PCR was performed: 1 cycle of 95°C for 3 min; 25 cycles each of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 1 cycle of 72°C for 5 min; and hold at 4°C. Clean-up of the PCR amplicon products to remove free primers and primer dimers was performed using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA). Fresh 80% ethanol was prepared prior to clean-up. Following amplicon clean-up, index PCR was performed to attach indexes to the amplicon PCR products. Dual-index primers were designed so that samples could be multiplexed in one MiSeq lane. Index PCR was performed as follows: 1 cycle of 95°C for 3 min; 8 cycles each of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 1 cycle of 72°C for 5 min; and hold at 4°C. Clean-up of the PCR index products was performed as above. All indexed amplicon concentrations were normalized and amplicons pooled into a single tube. The pooled library was checked for quality and quantified using an Agilent ScreenTape on the Agilent 2200 Tapestation (Agilent, Santa Clara, CA). The library was diluted and combined with a PhiX Control library (v3) (Illumina, San Diego, CA) at 10%. The library was sequenced on an Illumina v3 300PE MiSeq run, using standard sequencing protocols. Base calls were generated on-instrument during the sequencing run using the MiSeq Real Time Analysis (RTA 1.18.54) software and fastq generation; demultiplexing, adapter trimming, and quality filtering were performed by the MiSeq Reporter Software (2.4 and 2.5.1). The library was run on two lanes to increase the number of reads for each sample.

Sequence and statistical analyses

The resulting demultiplexed reads were processed using the QIIME 2 microbiome analysis package (Caporaso et al. 2010). Briefly, the paired end reads were joined together and Scikit-learn (Pedregosa et al. 2011) was used to taxonomically classify the resulting paired reads against the SILVA release 132 reference database (Pruesse et al 2007, Quast et al 2013, Yilmaz et al 2014) for 16S (prokaryote) analysis filtered at 99% identity. Reads not matching a reference sequence were removed from analysis. OTUs were assigned based on a database hit of 99% or greater sequence identity and taxonomy was assigned against the database. Core diversity analysis was used to perform α-diversity and rarefaction and β-diversity and rarefaction functions. Weighted and unweighted Unifrac distances (Lozupone and Knight 2005) were used to compute the between sample diversity which was visualized using principal coordinates analysis (PCoA) plots with Emperor (Vazquez-Baeza et al 2013).

RESULTS AND DISCUSSION

Study 1 - Evaluate the effectiveness of nutritional conditioning on tilapia feed efficiency and production

Results from the tank study suggest that restricting protein early in development (post-yolk sac absorption) enhances growth later in life (Figures 3, 4). Tilapia fry that were fed a diet containing 25% CP for 14 days and then transferred to a 38% CP diet had significantly higher lengths and weights relative to fry fed the control (48% CP) diets initially. Further, the growth of fry fed the 25% CP starter diet for 14 days that then remained on the 25% CP growout diet was not significantly different from either the controls or those transferred to the 38% CP diet indicating that it may be possible to restrict protein for up to 56 days provided they are switched to a growout diet. Survival rates were greatest (72%) in fry that received the 25% CP starter diet for only 7 days, however survival rates of fry that received the protein-restricted diet for 14 days (50%) were not significantly different from the control 48% protein diet (63% survival). Fry that were fed the 25% CP diet for 21 days had the lowest survival rate (28%) and thus it is recommended that tilapia be given a protein restricted starter diet for no longer than 14 days (Figure 5).

After obtaining positive results from the tank trials, we investigated whether this nutritional conditioning could also be applied to pond culture. Water quality was deemed suitable and within the range for fish culture throughout the study period and all parameters were relatively similar between treatments (Table 4). Contrary to what was observed in the tank study, tilapia fed a 40% CP diet initially (T3 and T4) were significantly larger after 93 days relative to those fed a 20% CP diet (Table 5). As tilapia are an omnivorous species, it is likely that natural food sources within the ponds negated any potential effects of the nutritional conditioning. Interestingly, the fish that were fed 40% CP initially and then switched to 20% CP (T3) had the highest survival, specific growth rates, and net production relative to all other groups (Table 5), leading to a significantly lower feed conversion ratio and higher net return (Figure 6). Thus, while nutritional conditioning per se may require nutrient restriction in tank systems to eliminate alternate food sources, it may still be possible to lessen feed costs for farmers in Bangladesh and other developing countries by reducing the amount of protein in growout diets. Further, despite growth and production being lowest in T1 (20% CP starter; 20% CP growout), this treatment had the second highest net profit and a similar benefit to cost ratio as T3 due to the lower cost of feed (Table 6). This suggests that providing low protein feeds throughout the culture period may be an acceptable option for farmers who have less money to invest and who can still market smaller fish. Introducing weekly fertilization as performed in this study could provide additional food sources and more diverse proteins to help compensate for the reduced levels in the formulated feed and may lead to enhanced growth.

Study 2 – Identify key factors (gene networks) associated with enhanced nutrient utilization in response to larval nutritional conditioning in tilapia

Nutritional conditioning is known to affect growth and immune function as well as enhance the uptake and utilization of specific nutrients in various vertebrates but little is known about the effects in fish and whether it can be applied to tilapia culture to enhance growth and feed efficiency. This study used a transcriptomic approach to identify changes in gene expression in the intestines of Nile tilapia fed a restricted 25% CP diet or a normal 48% CP diet for the first 14 days post-yolk sac absorption before being subjected to a 25% CP or 38% CP growout diet for 42 days. An RNA-seq analysis showed that tilapia fed 25% CP followed by 38% CP had 54 differentially expressed genes (DEG), those fed 25% CP followed by 25% CP had 1389 DEG, and those fed 48% CP followed by 25% CP had 105 DEG compared to the control diet (48% CP followed by 38% CP).

Gene Ontology (GO) analysis showed that in the 48% CP to 25% CP group, functions such as proteolysis (GO: 0006508), muscle contraction (GO: 0006936) and filament sliding (GO: 0030049), and immune responses (GO: 0006955) were enriched relative to the control (Table 7). The increase in genes associated with proteolysis (*klk2*, *map1b, tmprss15)* could indicate a restructuring of the intestine to provide amino acids for the production of more essential proteins given the decrease in dietary protein. This has been shown in neonatal pigs during weaning where providing a low protein diet suppresses protein synthesis (Deng *et al.*, 2009). One possibility for this restructuring could be to increase production of genes involved in muscle contraction such as myosin (*des, mylpf, myh11, myl3*) and actin (*acta1*) to help maintain normal intestinal function despite the lack of protein availability. In the 25% CP to 38% CP group, muscle contraction and filament sliding and immune response processes were also enriched compared to the control group (48% CP to 38% CP), in addition to cell signaling (GO: 0007267) and negative regulation of proteolysis (GO: 0045861)(Table 8). This likely indicates a compensatory mechanism wherein protein and muscle synthesis were enhanced due to the increase of dietary amino acid availability. Interestingly, a KEGG pathway analysis also showed upregulation of tight junctions (upregulated genes: *cldn4, mylpf, myh7*) in this group (25% CP to 38% CP). Low protein diets are known to decrease intestinal permeability to unwanted materials (Magnusson *et al.*, 1990) suggesting that this pathway may have been upregulated after the switch to the higher protein diet to prevent an increase in intestinal permeability. There were 1389 DEGs in fish subjected to an initial protein restricted diet and a low protein growout diet (25% CP to 25% CP group) relative to the control, 48% CP to 38% CP fish. Principal component analysis revealed the low protein group (25% CP to 25% CP) formed a distinct cluster separate from samples of the other three groups indicating vast differences in the intestinal transcriptomes of these fish, suggestive of broad physiological effects associated with a state of limited dietary protein (data not shown).

Study 3 – Identification of key microbial factors promoting increased nutrient absorption by enterocytes in the tilapia gastrointestinal tract

This investigation assessed how the gut microbial flora is altered by nutritional programming strategies and whether these changes could benefit the growth and health of tilapia. The establishment of beneficial gut flora to increase nutrient absorption is an emerging research focus in human biology and aquaculture science (Welker and Lim, 2011), and may serve to augment existing practices of sustainable feeding and reduction in environmental footprint. A total of 1,153,339 reads were obtained following sequencing of the V3 to V5 regions of 16S rRNA (prokaryote) after quality filtering of the reads.

A total of 23 prokaryotic phyla, 44 classes, 112 orders, and 202 families were associated with the tilapia used in this study (100% Bacteria, no Archeae). The dominant phyla identified overall from these samples belonged to the Proteobacteria (70.0%), Fusobacteria (12.1%), reads that could not be classified beyond the Kingdom Bacteria (11.1%), Actinobacteria (3.1%), and Bacteroidetes (2.1%) (Figure 7). The proportions of the identified microbes varied between treatments and between collection time points. Bacteria from the class Fusobacteriaceae were most abundant in the fecal material of Nile tilapia used in our previous study of alternate feeding strategies in tilapia culture. *Cetobacterium somerae* had the highest number of reads or abundance of Fusobacteria found in this study. This species has been isolated from the intestinal tract of cultured freshwater fish (Tsuchiya et al. 2008) and thus may play an important role in the health of these fishes.

OTUs that made up the greatest proportion of the Proteobacteria belong to the class Gammaproteobacteria. These include bacteria in the genus *Aeromonas* (10.1% overall), genus *Rheinheimera* (4.6% overall), genus *Shewanella* (1.3% overall), family Burkholderiaceae (4.7% overall), genus *Cellvibrio* (1.4% overall), genus *Pseudomonas* (1.7% overall), and genus *Enterovibrio* (14.0% overall). Microbes found in the genus *Aeromonas* include many that are opportunistic pathogens of fishes. One OTU observed belongs to the species *A. hydrophila*, a major pathogen in freshwater fishes causing ulcers, “fin and tail rot”, and hemorrhagic septicemia (Subashkumar et al. 2007) along with antibiotic resistant strains found in Mozambique tilapia (*Oreochromis mossambicus*; Son et al. 1997). This OTU was found in our baseline samples and in our samples taken at day 14 whether fed the 25% or 48% initial diet but was found at proportions of less than 1% in these treatments. It was not observed in any of the later treatments. Organisms of the genus *Rheinheimera* include halotolerant freshwater and marine oligotrophs, mostly aquatic in nature (Baek and Jeon 2015). *R. aquatica* is an antimicrobial producing microorganism found in freshwater culture ponds (Chen et al. 2010; Sun et al. 2016). Bacteria of the genus *Shewanella* are normal components of surface floral of fish and have been implicated in fish spoilage (Adams and Moss 2008). Bacteria in the family Burkholderiaceae can be found in a wide variety of niches, from soil and water to close associations with plants and animals (Coenye 2014). They also have a wide variety of functions including nitrogen fixation but many are highly pathogenic (Coenye 2014). We were not able to identify members of this family to the species level in this study but none of the genera identified include those of common pathogens. Members of the genus *Cellvibrio* are mainly cellulolytic organisms that produce xylanases and other hydrolases that break down cellulose and other polysaccharides (Wu and He 2015, Xie et al 2017). Of the members identified to the species level in this study within the genus *Pseudomonas*, *P. peli* is a nitrifying bacterium that has been found associated with aquaculture ponds, tanks, and aquaria (Vanparys et al 2006). Based on this, it is not unfounded that this organism was identified in this study. The genus *Enterovibrio* also includes species that have been found associated with fishes. One of these (not identified in the current study) has been isolated from the gut of larval turbot (*Scophthalmus maximus*; Thompson et al. 2002). This species *E. norvegicus*, was found in the guts of healthy cultured fish in Norway and may improve larval turbot survival and growth.

Alpha diversity measures are indices of the diversity within a community. The Observed OTUs index is commonly used to estimate the operational taxonomic unit (OTU) richness within a community and is based on the amount of unique OTUs in each sample. Alpha diversity and rarefaction curves were determined for all treatments evaluated. The treatment initially fed on a restricted diet for 14 days followed by the restricted growout diet had the highest measure of OTU richness of all treatments measured (Figure 8). There was no difference in OTU richness between fish fed high or restricted protein starter diets for 14 days. However, fish that had been fed the 48% CP diet initially showed a large decrease in OTU richness after 7 days on the growout diet, regardless of the protein content, while those fed the restricted 25% CP diet retained high OTU richness on both the 25% and 38% CP growout diets (Figure 8). Beta diversity, as illustrated by Principle Coordinates Analyses (PCoA) plots, is a measure of the microbial diversity between treatments. Our analyses indicate no differences in sample microbial communities between treatments (Figure 9). Principal Coordinate 1 explained 15.60% of the variability between samples, while principal coordinate 2 explained 14.15% of the variability.

There were 45 novel bacterial OTUs (15.7%) found in the fish fed the 25% protein restricted diet for 14 days compared to the fish fed the 48% protein diet for 14 days, and 124 novel bacterial OTUs (43.2%) found in the fish fed the 48% protein initial diet for 14 days versus those fed the 25% protein restricted initial diet for 14 days. Together they shared 118 (41.1%) of their bacterial OTUs (Figure 10). Many of these novel organisms were only identified to the family or genus taxonomic level so a complete idea of the roles these microbes may play in the tilapia fry is unknown. At the time of sampling (14 days after the start of the study), the fish fed the 25% protein diet were 0.093±0.091 g and 14.04±4.32 mm TL while the fish fed the 48% protein diet were 0.079±0.031 g and 13.87±2.89 mm TL (Figures 3, 4). Two organisms of notable significance and concern preliminarily identified in the analysis of the fry fed the 25% protein diet but not the 48% protein diet and also in the baseline samples were *Mycobacterium sp*. and *Flavobacterium columnare*. Both of these species are highly pathogenic in fishes and tend to proliferate in infected culture systems. Mycobacteriosis is common particularly in intensive aquaculture systems. It causes chronic infections leading to weight loss, scale loss, ulcers, and occasionally deep hemorrhagic skin lesions and is known to lead to zoonotic infections (Francis-Floyd 2011, Gauthier and Rhodes 2009). *F. columnare* is the causative agent of columnaris disease in fish. It is a serious disease of freshwater fishes. It is a highly pathogenic organism with infected fish showing symptoms of skin and fin erosion and lesions and gill necrosis, often leading to mortalities (Declercq et al. 2013). In our studies neither of these organisms were identified in any of the later samples.

CONCLUSION

It is estimated that 60-80% of total production costs for culturing tilapia is attributable to feeds, with high quality protein being the most costly ingredient. This study is the first to demonstrate that providing a reduced protein diet for the first 14 days post-yolk sac absorption can lead to enhanced growth later in life when fingerling tilapia are cultured in tank systems. A transcriptome analysis of tilapia intestinal tissue showed differential expression of genes involved in proteolysis and muscle function between groups receiving different levels of protein which may indicate compensatory mechanisms for maintaining function during protein restriction or enhancing muscle synthesis when dietary protein levels are increased. We also observed an increased species diversity of microbes in fish fed a protein-restricted diet which may enhance the overall health of the animals. Despite a robust nutritional conditioning response seen in tank trials, early protein restriction was ineffective in enhancing growth of fingerlings in pond culture, likely due to the abundance of natural protein sources within the ponds (eg. plankton, algae, etc.) and inadequate early protein restriction. Future studies should test whether early restriction of protein under more controlled conditions in hatcheries yields greater growth rates and yield of tilapia during growout in ponds. Nonetheless, our results suggest that it may still be possible to reduce feed costs for fish farmers in Bangladesh by reducing the protein content of the diet during the growout phase rather than immediately following yolk-sac absorption as the treatment group provided with a 40% CP diet followed by a 20% CP diet had the greatest growth, production, and net returns relative to all other treatments.

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TABLES AND FIGURES

Table 4. Water quality parameters (mean ± SD) for the pond trials in Study 1. Values with different letters are significantly different (One-way ANOVA; $P < 0.05$). TDS = total dissolved solids.

	Treatment 1	***Treatment 2***	***Treatment 3***	***Treatment 4***
Temperature (°C)	28.08±2.58	28.05±2.54	28.40±2.03	28.28±1.90
Transparency (cm)	11.58±0.96^{a}	14.58±1.67^{b}	12.83±0.58^{c}	13.53±0.56^{d}
TDS (mg/l)	58.10±7.60^{a}	61.65±6.50^{a}	67.94±21.57^{a}	82.26±20.43^{b}
Alkalinity (mg/l)	45.88±7.70	48.15±7.87	46.30±5.02	49.76±3.95
pH	8.46±1.31	8.25±1.23	8.57±1.22	8.57±1.32
Dissolved Oxygen (mg/l)	5.95±1.18^{a}	5.22±1.06^{b}	5.19±1.04^{b}	5.45±1.32^{ab}
Nitrate (mg/l)	0.02±0.01^{a}	0.02±0.01^{a}	0.03±0.01^{b}	0.02±0.01^{a}
Nitrite (mg/l)	0.033±0.01^{a}	0.05±0.02^{b}	0.03±0.02^{a}	0.03±0.02^{a}
Ammonia (mg/l)	0.55±0.33	0.49±0.29	0.42±0.33	0.49±0.18
Phosphate (mg/l)	2.05±0.55^{a}	1.52±0.29^{b}	1.60±0.36^{b}	1.58±0.36^{b}
Chlorophyll-a (µg/l)	210.8±20.3^{a}	171.8±13.8^{b}	191.6±13.6^{c}	181.4±12.8^{d}

Table 5. Growth and production yield (mean ± SD) of tilapia (*O. niloticus*) for the pond trials in Study 1. Values with different letters are significantly different (One-way ANOVA; $P < 0.05$). SGR = specific growth rate; FCR = feed conversion ratio.

	Treatment 1 20%:20% CP	***Treatment 2 20:40% CP***	***Treatment 3 40:20% CP***	***Treatment 4 40:40% CP***
Initial Weight (g)	0.24 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.24 ± 0.00
Final Weight (g)	50.4 ± 4.62^{a}	61.6 ± 3.86^{b}	120.0 ± 5.14^{c}	81.5 ± 5.10^{d}
Weight Gain (g)	50.2 ± 4.62^{a}	61.4 ± 3.86^{b}	120.1 ± 5.14^{c}	81.3 ± 5.10^{d}
SGR (%/day)	1.50 ± 0.05^{a}	1.55 ± 0.08^{b}	1.67 ± 0.05^{c}	1.48 ± 0.05^{a}
FCR	0.58 ± 0.01^{a}	0.45 ± 0.01^{b}	0.38 ± 0.01^{c}	0.48 ± 0.01^{d}
Survival Rate (%)	90.5 ± 1.31^{a}	92.6 ± 1.53^{a}	97.7 ± 1.05^{b}	93.3 ± 1.47^{a}
Net Yield (kg/ha)	8424 ± 263^{a}	10095 ± 268^{b}	11075 ± 251^{c}	10525 ± 257^{d}
Total Yield (kg/ha)	8900 ± 263^{a}	10571 ± 269^{b}	11500 ± 251^{c}	10950 ± 257^{d}

Table 6. Economic analysis of tilapia (*O. niloticus*) raised in ponds (Study 1). Values with different letters are significantly different (One-way ANOVA; $P < 0.05$).

	Treatment 1	***Treatment 2***	***Treatment 3***	***Treatment 4***
Expenditure (BDT/pond)				
Fingerlings	30,875	30,875	30,875	30,875
Feed	348,888[a]	486,111[b]	486,111[b]	523,333[c]
Lime	7,547	7,547	7,547	7,547
Fertilizers	24,013	24,013	24,013	24,013
Pond Lease	28,816	28,816	28,816	28,816
Labour	10,000	10,000	10,000	10,000
Operational Cost*	33,760	44, 052	44, 052	46,844
Total Expenditure	483,899	631,414	631,414	671,428
Income				
Gross Return (BDT/pond)				
Net Return (BDT/pond)				
Gross Return (BDT/ha)	712,000[a]	845,686[b]	920,000[c]	876,000[d]
Net Return (BDT/ha)	228,101	214,272	288,586	204,572
BCR (Benefit Cost Ratio)	1.47	1.34	1.46	1.30

*Operational cost is considered as 7.5% of total cost (ADCP, 1983).

Table 7. Significantly enriched (p<0.10) gene ontology (GO) biological processes and Kegg pathways associated with human homologues of genes that were significantly differentially expressed (FDR p-value <0.05) in the anterior intestine of fish fed a 48% protein diet for 14 days and a 25% protein diet for 42 days relative to the control fish (48% protein for 14 days, then 38% protein for 42 days)

Enriched GO Processes and Kegg Pathways 48% to 25% Protein			
GO Number	**Biological Process**	**P-Value**	**Differentially Expressed Genes**
GO:0006936	muscle contraction	0.0044319	DES, ACTA1, MYH11, MYLPF
GO:0030049	muscle filament sliding	0.0061573	DES, ACTA1, MYL3
GO:0006955	immune response	0.0095619	CCL3, CHIA, MYLPF, IL2RG, MR1, HLA-F
GO:0006508	proteolysis	0.0188999	KLK2, MEP1B, CAPN2, CAPN3, TMPRSS15, CAPN1
GO:0030259	lipid glycosylation	0.0214804	B4GALNT2, B4GALNT1
GO:0022617	extracellular matrix disassembly	0.0231663	KLK2, CAPN2, CAPN1
GO:0002674	negative regulation of acute inflammatory response	0.0245119	APCS, INS
GO:0006486	protein glycosylation	0.0478591	ST3GAL1, B4GALNT2, B4GALNT1
GO:0001574	ganglioside biosynthetic process	0.0543244	ST3GAL1, B4GALNT1
GO:0045861	negative regulation of proteolysis	0.0630925	KNG1, INS
GO:0051092	positive regulation of NF-kappaB transcription factor activity	0.0638669	CARD14, INS, CAPN3
GO:0055010	ventricular cardiac muscle tissue morphogenesis	0.0746595	MYL3, PKP2
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	0.0889214	MR1, HLA-F
KEGG Number	**Pathway**	**P-Value**	**Differentially Expressed Genes**
hsa00604	Glycosphingolipid biosynthesis - ganglio series	0.061196272	ST3GAL1, B4GALNT1

Table 8. Significantly enriched ($p<0.10$) gene ontology (GO) biological processes and Kegg pathways associated with human homologues of genes that were significantly differentially expressed (FDR p-value <0.05) in the anterior intestine of fish fed a 25% protein diet for 14 days and a 38% protein diet for 42 days relative to the control fish (48% protein for 14 days, then 38% protein for 42 days)

Enriched GO Processes and Kegg Pathways 25% to 38% Protein			
GO Number	**Biological Process**	**P-Value**	**Differentially Expressed Genes**
GO:0030049	muscle filament sliding	0.0000004	TNNT3, DES, ACTA1, MYL3, MYH7
GO:0006936	muscle contraction	0.0006582	DES, ACTA1, MYLPF, MYH7
GO:0006942	regulation of striated muscle contraction	0.0143819	TNNT3, MYL3
GO:0006955	immune response	0.0293124	CHIA, MYLPF, MR1, HLA-F
GO:0002026	regulation of the force of heart contraction	0.0301281	MYL3, MYH7
GO:0048545	response to steroid hormone	0.0316893	ACTA1, SST
GO:0045861	negative regulation of proteolysis	0.0332481	INS, CSTB
GO:0003009	skeletal muscle contraction	0.0379101	TNNT3, MYH7
GO:0055010	ventricular cardiac muscle tissue morphogenesis	0.0394592	MYL3, MYH7
GO:0032148	activation of protein kinase B activity	0.0410060	INS, TXN
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	0.0471691	MR1, HLA-F
GO:0007267	cell-cell signaling	0.0624496	INS, TXN, SST
GO:0060048	cardiac muscle contraction	0.0699428	MYL3, MYH7
GO:0007519	skeletal muscle tissue development	0.0789048	MYL3, MYLPF
GO:0007586	digestion	0.0965798	CHIA, SST
KEGG Number	**Pathway**	**P-Value**	**Differentially Expressed Genes**
hsa04530	Tight junction	0.0263817	CLDN4, MYLPF, MYH7
hsa04940	Type I diabetes mellitus	0.0762614	INS, HLA-F

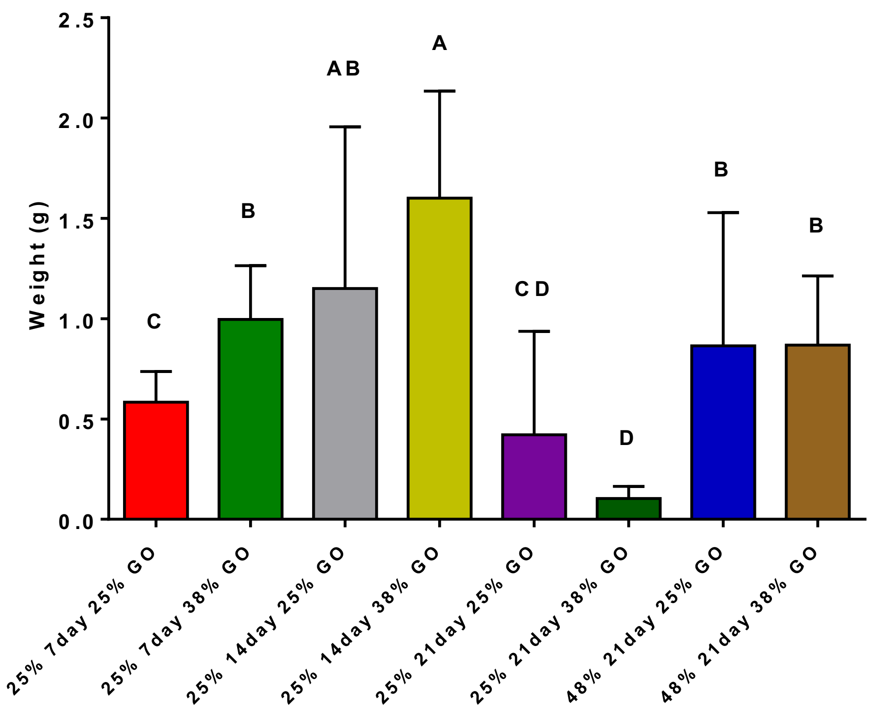

Figure 3. Final weights of Nile tilapia (*O. niloticus*) after 56 days culture in tank trials from Study 1. Data are means ± SD. Values with different letters are significantly different (One-way ANOVA; P < 0.05; GO, growout).

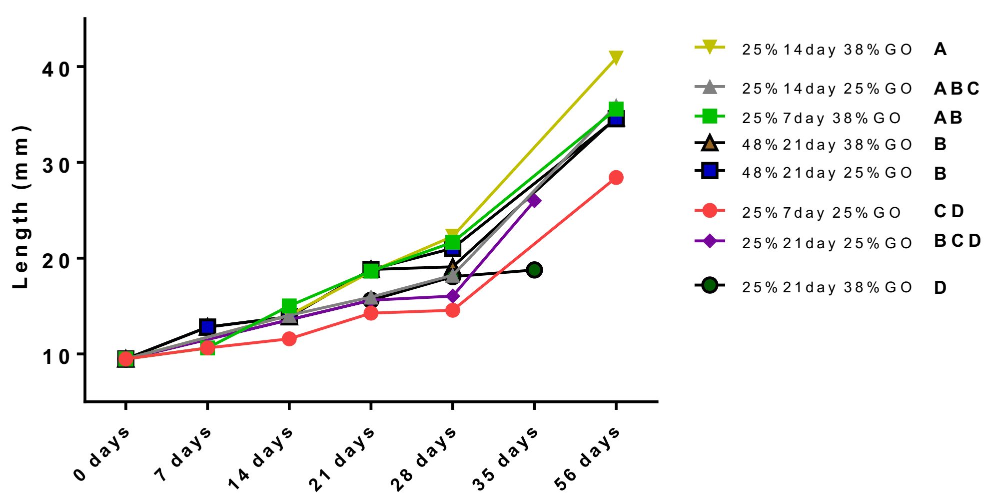

Figure 4. Mean lengths of Nile tilapia (*O. niloticus*) over the 56-day culture period for the tank trials in Study 1. Values with different letters are significantly different (Two-way ANOVA; P < 0.05). Error bars have been excluded for clarity.

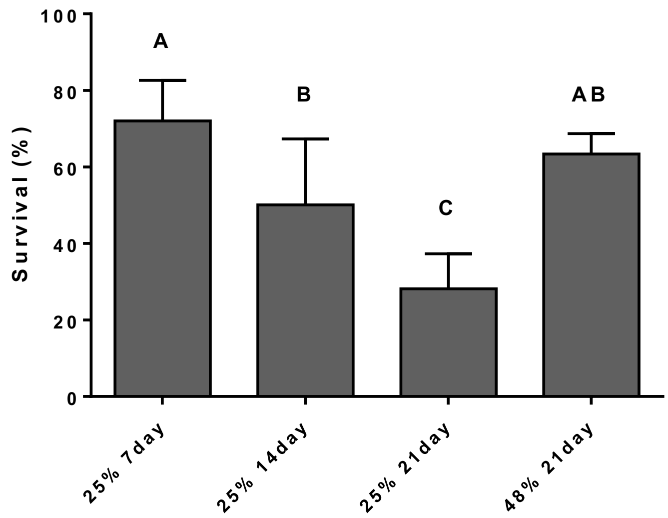

Figure 5. Survival rates (%) of Nile tilapia (*O. niloticus*) after 56 days in tank trials from Study 1. Data are means ± SD. Values with different letters are significantly different (One-way ANOVA; $P < 0.05$).

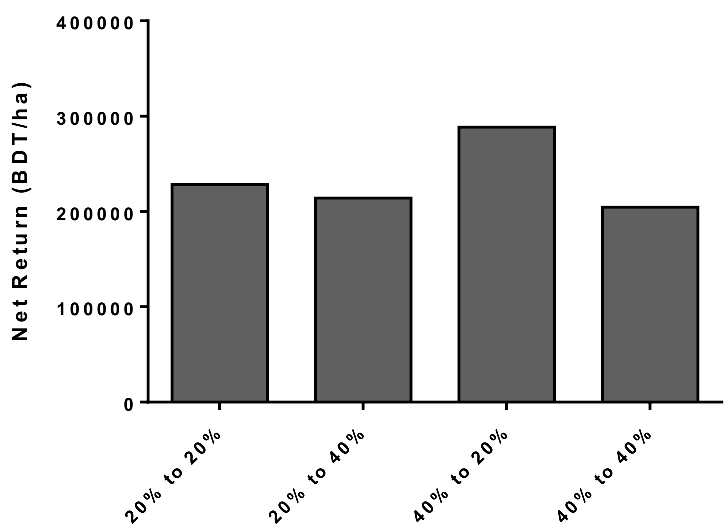

Figure 6. Net returns (BDT/ha) for Nile tilapia (*O. niloticus*) raised in ponds for Study 1.

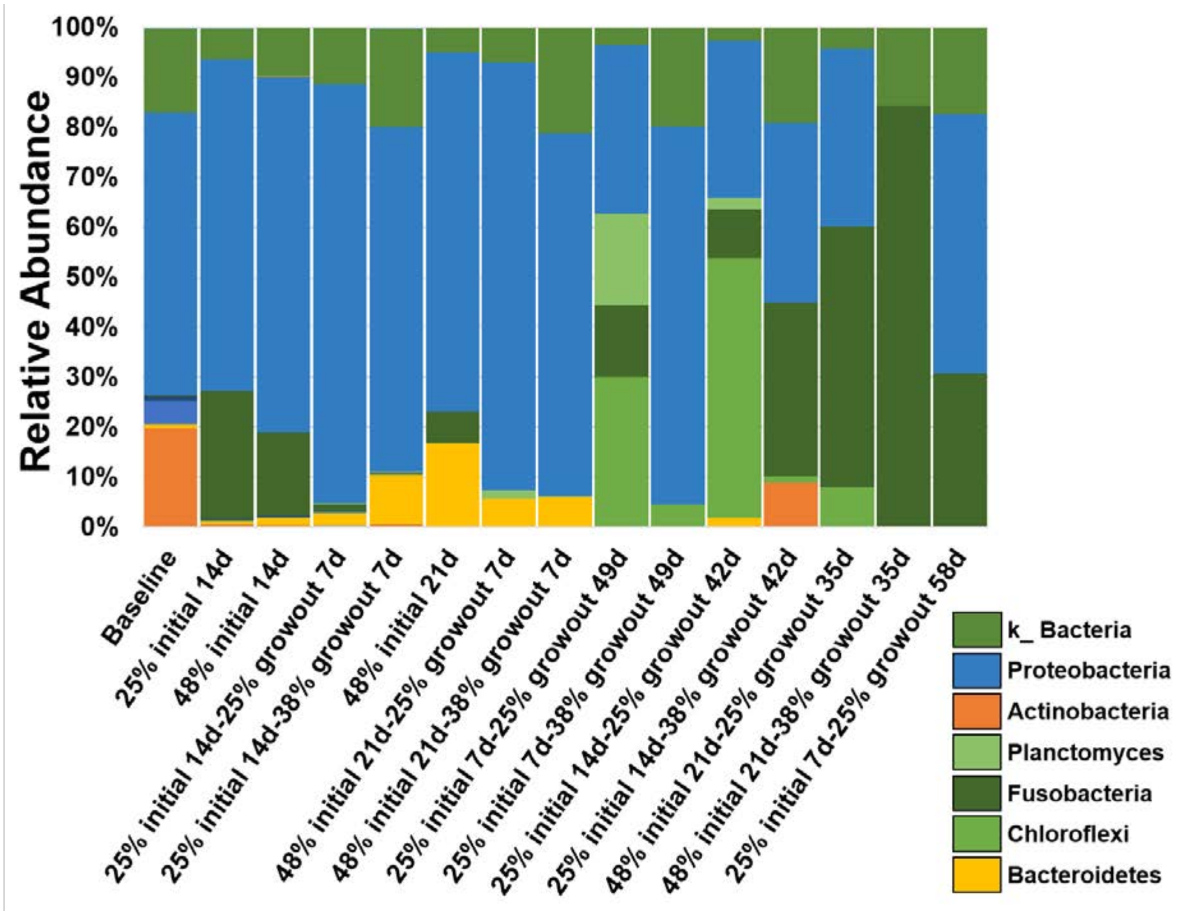

Figure 7. Relative abundance of phyla identified from Nile tilapia fry after feeding with diets containing restricted or normal protein levels. k_Bacteria = Kingdom Bacteria; these OTUs could not be identified beyond the Kingdom taxonomic level.

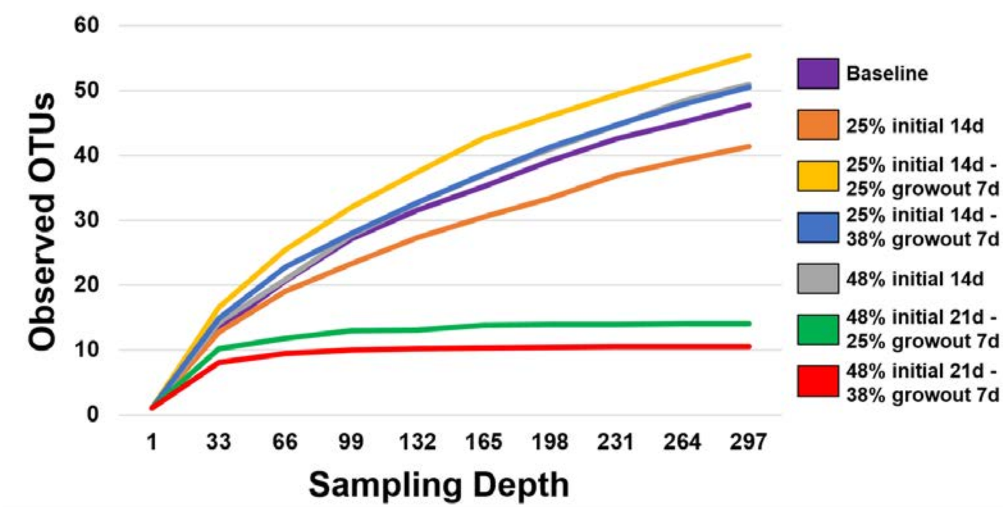

Figure 8. Alpha rarefaction plot to estimate species richness within bacterial communities found in Nile tilapia fry fed varying protein level diets.

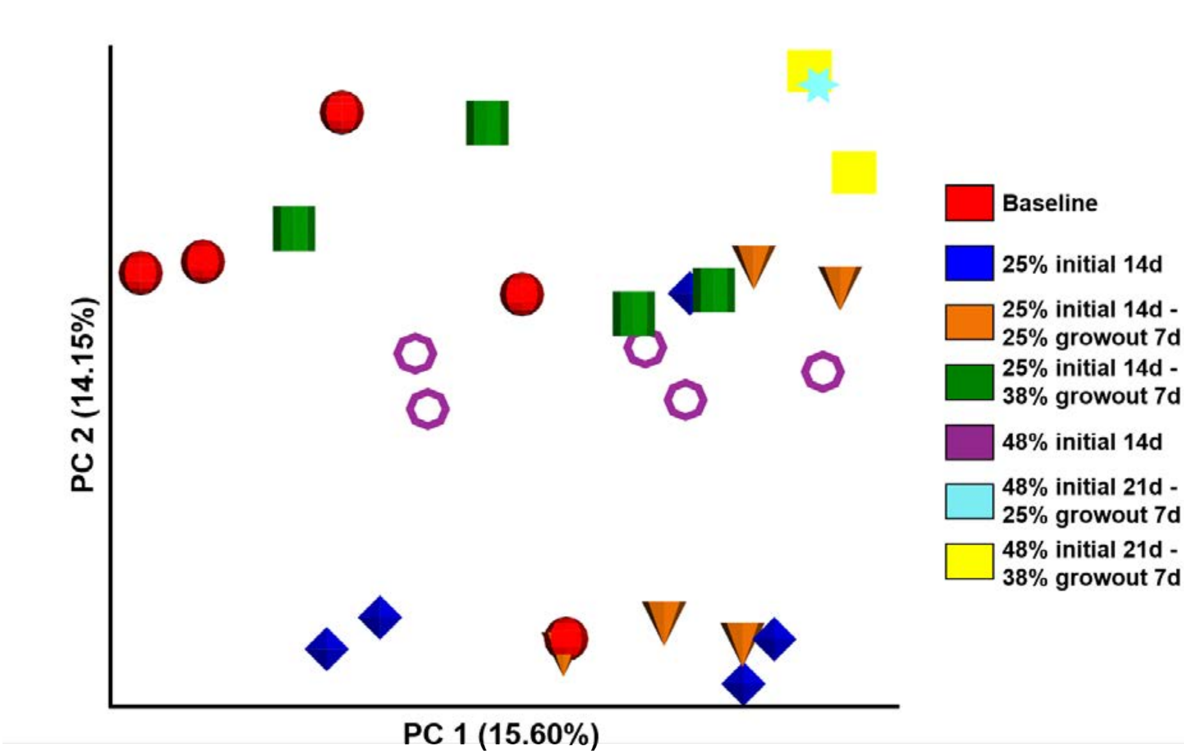

Figure 9. Beta diversity analysis of pair-wise differences in microbial communities in Nile tilapia fry fed varying protein level diets.

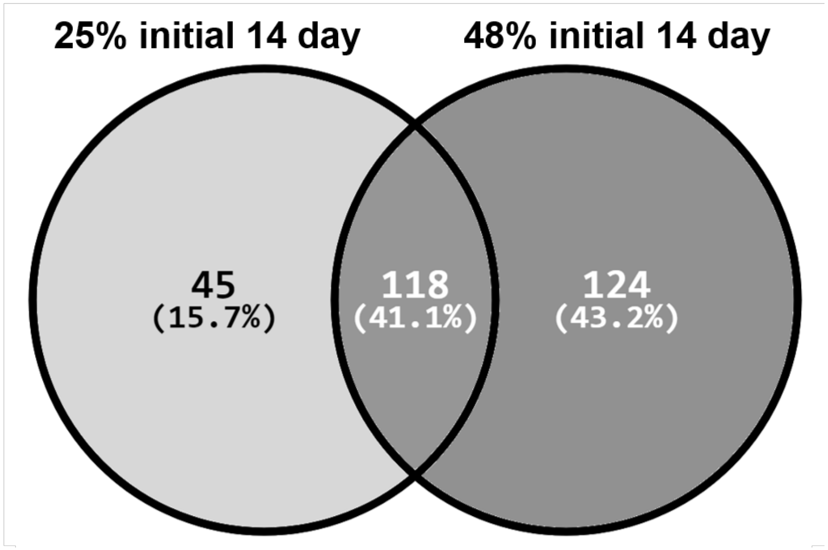

Figure 10. The numbers of novel OTUs identified when comparing Nile tilapia fry fed a 25% protein initial diet for 14 days and those fed a 48% protein initial diet for 14 days.

INCREASING PRODUCTIVITY OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) THROUGH ENHANCED FEEDS AND FEEDING PRACTICES

Sustainable Feed Technology and Nutrient Input Systems/Experiment/16SFT03PU

Sebastian W. Chenyambuga[1], Nazael A. Madalla[1], Renalda Munubi[1] and Kwamena Quagrainie[2]

[1]*Sokoine University of Agriculture, Morogoro, Tanzania.*
[2]*Purdue University, USA*

ABSTRACT

Productivity of farmed fish in Tanzania is low mainly due to poor feeds and feeding practices. Small-scale fish farmers cannot afford the price of fishmeal and soybean meal that are used as protein sources in fish diets. This study was conducted to determine appropriate inclusion levels of substituting fishmeal and soybean meal with a combination of Moringa leaf meal and housefly maggot meal as sources of protein in Nile tilapia diets. In addition, the study aimed at determining the most suitable substrate for production of housefly (*Musca domestica*) maggots and the appropriate feeding strategy for minimizing feed cost in Nile tilapia production. Three experiments were conducted. In the first experiment a total of five substrates (cow dung, chicken manure, pig manure, cattle offal and kitchen leftovers) were tested for production of housefly maggots. Also, the effects of quantity of manure and the age at which housefly maggots are harvested on maggot yields were assessed. In the second experiment a feeding trial was carried out to evaluate the effect of substituting fishmeal and soybean meal with a mixture of Moringa leaf meal and housefly maggot meal on growth performance of sex reversed Nile tilapia (*Oreochromis niloticus*). Eight diets were formulated and each diet contained about 30% crude protein. Diet one (D_1) was a control diet and contained only fishmeal as the source of protein. Diet D_2 was another control diet, but based on soybean meal as the main source of protein. Diets D_3, D_4, D_5, D_6 and D_7 contained various combinations of housefly maggot meal and Moringa leaf meal while diet D_8 contained only housefly maggot meal as sources of protein. The feeding experiment was conducted using 24 plastic tanks, each with capacity of 30 litre, in an indoor semi-recirculation system. Each tank was stocked with 10 Nile tilapia fingerlings with mean initial weight of 1.17 ± 0.04 g. The fish were fed to apparent satiation. Water temperature, pH and dissolved oxygen (DO) were measured weekly. Body weight was measured once per week during the experimental period. The experiment took 56 days. In experiment 3 the best diet identified in experiment 2 was used. Using this diet four feeding strategies were tested i.e. daily feeding at a level of 2.5% of fish weight (T_1), daily feeding at a level 5% of fish weight (T_2), feeding one day at 2.5% followed by one day feeding at 5% of fish weight (T_3) and feeding two days at 2.5% followed by two days feeding at 5% of fish weight (T_4). Nile tilapia fingerlings with average body weight of 10.43 g were stocked in outdoor concrete tanks (each 4.5 m^2 in size and 1 m deep) at a stocking rate of 10 fingerlings per tank. The experiment was conducted for 70 days. Final body weight, weight gain, daily weight gain, specific growth rate, daily feed intake, feed conversion ratio (FCR), protein efficiency ratio, feed costs, revenue and gross margin were computed.

Results show that the type of substrate influenced the yield of maggots ($p \leq 0.05$). The use of cattle offal resulted in significantly higher maggot yield ($p \leq 0.05$) compared to the other substrates. The quantity of maggots produced from chicken manure (40.83 ± 0.67 g/kg) was higher ($p \leq 0.05$) than that obtained from pig manure (27.25 ± 1.29 g/kg), cattle manure (21.57 ± 0.21 g/kg) and kitchen leftovers (27.24 ± 0.33 g/kg). Increasing the quantity manure from 6 to 12 kg increased the yield of maggots from 54.49 ± 1.43 to 74.72 ± 1.43 g/kg of manure. Maggots harvested five days after hatching showed significantly higher yield (72.23 g/kg of manure) than those harvested four and three days after hatching. Fishmeal had the highest crude protein content (60.59%), followed by maggot meal (46.96%). In the second experiment the type of diet had significant effect ($p \leq 0.05$) on body weight gain, average daily weight gain and specific growth rate. Fish fed diet D_1 had the highest body weight gain (3.32 ± 0.25 g), average daily weight gain (0.059 ± 002 g/day) and specific growth rate (2.91%/day), followed by those on D_8 and D_6. Fish fed diet D_5 had the lowest body weight gain (2.07 ± 0.23 g) and average daily weight gain (0.037 g/day). Feed conversion ratio (FCR) was lower on fish fed diet D_1 (2.34 ± 0.06) and D_6 (2.46 ± 0.06), but was higher on those fed diet D_7 (2.84 ± 0.06) and D_4 (2.83 ± 0,06). The type of diet had no significant effect ($p > 0.05$) on protein efficiency ratio. Fish fed diet D_4, D_6, D_7, D_1 and D_8 had significantly higher survival rate compared to those on diet D_2 and D_5.In the third experiment, Nile tilapia reared under T_3 (alternating between feeding levels of 2.5 and 5% of body weight daily) showed higher ($p \leq 0.05$) mean weight gain (93.92 g), average daily gain (1.60 g/day), specific growth rate (5.13%/day), estimated yield (12,969.60 kg/ha/year), revenue (TZS 116,726,499.00 per year) and gross margin (TZS 78,480,026.67 per year) than of those under T_1, T_2 and T_4. The highest feed cost (TZS 25,974,666.67) and total cost (TZS 41,585,77.78) were observed on fish under T_2 (daily feeding at 5% of body weight) while the lowest were found on T_1 (daily feeding at 2.5% of body weight). It is concluded that chicken manure is a better substrate than cattle manure and pig manure for production of maggots. Housefly maggot meal can be used to replace fish meal in the diets without affecting the growth performance of Nile tilapia. Housefly maggot meal has higher protein content and promotes better growth performance than soybean meal. Furthermore, alternating feeding levels of 2.5 and 5% of fish weight daily is the best feeding strategy and can be used to increase the profitability of aquaculture enterprise.

INTRODUCTION

Aquaculture is one of the world's fastest growing animal producing sector with an average growth rate of 8.8%, outpacing capture fisheries (1.2%) and terrestrial farmed meat production (2.8%) (FAO, 2014). Aquaculture accounts for almost half of the world's fish food supply (FAO, 2014). Therefore, aquaculture offers a great potential for food security, poverty alleviation and enhanced trade and economic benefits (ADB, 2005). Aquaculture expansion in Asian countries such as Bangladesh and Thailand has led to enhanced food security among adopters and the population at large (Pant *et al*., 2004; De Silva and Davy, 2010; Jahan *et al*., 2010; Lazard *et al*., 2010). Furthermore, fish are a good source of animal-protein containing essential nutrients of high bioavailability which are found in limiting amounts in most human diets. These nutrients include essential amino acids, essential fatty acids, minerals and vitamins. Fish is a good source of long-chain omega-3 fatty acid docosahexaenoic acid (DHA) that is important for optimal brain and neurodevelopment in children and eicosapentaenoic acid (EPA) that improves cardio-vascular health. Thus, improving fish production from aquaculture will increase the intake of these important nutrients required in a healthy diet and thus reduce the problem of malnutrition.

Despite its potential for improving livelihoods, aquaculture has never developed to a significant extent in Tanzania. Chenyambuga *et al.* (2014) reported tilapia productivity of 5,312 kg ha^{-1} yr^{-1}. This low productivity is mostly attributed to poor feeds and feeding practices. Feeding of fish cultured in ponds of small-scale farmers depends on natural food in the ponds produced by irregular application of inadequate manure. In addition, fish farmers in rural areas provide maize bran, kitchen leftovers and green vegetables/weeds as supplementary feeds. These feeds are of poor quality and when fed as sole diets results into slow growth and low yield of fish at harvest. Elsewhere, it has been shown that with proper feeds and feeding practices, it is possible to attain yields of up to 19,000 kg ha^{-1} yr^{-1} (Hasan and New, 2013). To achieve this high level of production, it is important to develop good quality diets for use in hatcheries, nurseries and grow-out ponds. For many decades, fishmeal and soybean have been used as the main sources of protein in fish feeds due their high quality in terms of amino acid profile and palatability (El-Sayed, 1999; El-Saidy and Gaber, 2002). However, fish farmers in Tanzania cannot afford these two sources essential to meet protein requirement required for high productivity in farmed fish. Thus, there is a need to identify more affordable alternative sources of proteins.

Plant protein sources such as *Moringa oleifera* leaf meal can replace fishmeal, either partially or totally, in practical Nile tilapia diets (Afuang *et al*., 2003). Our previous study showed that a diet containing a mixture of Moringa leaf meal and sunflower seed cake in equal proportions can promote higher growth rate of Nile tilapia, even better than soybean meal (Shigulu, 2012; Kitojo, 2013). Moreover, insects and other invertebrates have been shown to be cheaper sources of animal protein in tilapia diets (Omoyinmi and Olaoye, 2012). These invertebrates are abundantly available because of their short life cycle and ability toproduce large numbers and high biomass within a short time. Our previous study showed that diets containing housefly maggot meal as source of protein promoted higher growth rate of Nile tilapia than cotton seed cake-based diets (Ally, 2015). Thus, the diets based on Moringa leaf meal and maggot meal can be used as alternative to fishmeal and soybean meal as sources of protein, since they have high crude protein content and are abundantly available and affordable to small-scale farmers. However, suitable combination of Moringa leaf meal and maggot meal in Nile tilapia diets have not been established. Likewise, there was a need to determine appropriate feeding practice in terms of feed amount and feeding frequency of fish cultured under semi-intensive production system. Inappropriate feeding practices such as overfeeding can result into feed wastage leading to water quality deterioration and increased production costs (Abdelghany and Ahmad, 2002; Deyab and Husseinm 2015; Ali*et al*., 2016). On the other hand, underfeeding can result into stunted growth and prolonged production cycle, which altogether cause a loss in fish farming enterprise. Therefore, development of appropriate feeding strategies is imperative in order to optimize feed efficiency by reducing feed wastage and deterioration of water quality and ensure profitability. The overall objective of this study was to develop a good quality and cheap diet for Nile tilapia based on Moringa leaf meal and housefly maggot meal. Specifically, the study aimed at determining (1) suitable technique for mass production of housefly maggots, (2) appropriate inclusion levels of a combination of Moringa leaf meal and housefly maggot meal in Nile tilapia diets and (3) appropriate feeding strategy for minimizing feed cost in Nile tilapia production.

METHODOLOGY

Study location and Sampling procedure

The experiment was conducted at Magadu fish farm, Sokoine University of Agriculture (SUA), Morogoro. Sokoine University of Agriculture is located between latitude 6 - 7^{o}S and longitude 37 - 38^{o}E at an altitude of about 500 - 600 m above sea level. The area receives an average annual rainfall of between 600 and 1000 mm. The climate is characterized by bimodal rainfall patterns, with short rains starting in November and ending in December and long rains starting in March and ending in May. The temperature ranges from 25^{0} to 30^{0}C.

Experiment 1: Determination of the most suitable substrate for maggot production

This experiment involved designing an appropriate method and determining the most suitable substrate for production of housefly maggots.

Designing of method for production of housefly maggot

Fifteen plastic containers were designed and used for production of maggots. Each had 40 cm diameter and 21.5 cm height (Plate 1). The container consisted of two chambers: the top and the bottom chambers. The top chamber is the culture chamber in which substrates were placed. This chamber was separated from the bottom chamber using a 2 mm plastic mesh to allow dropping of maggots into the harvesting chamber. The dimensions of the culture chamber were 7 cm height and 40 cm diameter. The bottom chamber was the harvesting chamber from which the maggots were collected. The dimensions were 40 cm diameter and 14.5 cm height. The base of this chamber was covered by a lid which can easily be opened during collection of maggots.

Plate 1: The culturing containers

Determining suitable substrate for maggot production

A total of five substrates (cow dung, chicken manure, pig manure, cattle offal and kitchen leftovers) were tested for production of housefly maggots. The experiment was conducted for a period of 21 days. The treatments were cow dung (treatment one - $\mathbf{T_1}$), chicken manure (treatment two - $\mathbf{T_2}$), pig manure (treatment three - $\mathbf{T_3}$), cattle offal (treatment four - $\mathbf{T_4}$) and kitchen leftovers (treatment five - $\mathbf{T_5}$). The treatments were allocated randomly to 15 culture containers shown above and replicated three times. The substrates were put in an air-tight plastic bucket with capacity of ten (10) liters for 24 hours so as to kill fly eggs or maggots if any. A total of 2.5 kg of each substrate (cattle, pig and chicken manures; cattle offal and kitchen leftovers) was put in the culture chamber of the container. Then, 250 g of mixture of blood, small pieces of meat debris and rotten eggs (as housefly attractant) was spread over substrate in each culture chamber. Each culture container was half covered with polythene sheet and a lid, leaving space for houseflies to get in for laying eggs. Substrates were exposed for 7hrs (11:30 am to 05:30 pm) to allow oviposition by the flies. Few perforations were made through the nylon and lids in order to allow aeration. Hatched larva were seen two days after oviposition.

Maggots were dropping from the culture chamber into the harvesting chamber. Harvesting of maggots was done once on the fourth day after oviposition. Prior to harvesting, temperature in each substrate was measured. Collection of maggots, from each harvesting chamber was done by opening the base (lid) of the bottom chamber of the culture container. Collected maggots were thoroughly washed with water and then blanched in hot water at 70 ^{o}C for 10 seconds. Thereafter weighed using digital weighing balance and then dried in oven at 60^{o}C for 48 hours for determination dry matter content.

Another study was conducted to evaluate the effects of quantity of manure and the age at which housefly maggots are harvested on maggot yields. Chicken manure was used as the substrate for culturing the housefly maggots and two levels of manure were used (6 kg and 12 kg), each replicated three times. The manure were spread onto culture chamber and exposed to flies to lay eggs which hatched into maggots. The maggots were harvested at three different ages after hatching i.e. on day three, day four and day five. The harvested maggots were cleaned with water, blanched in hot water at 70^{o}C for 10 sec, weighed and then dried in the oven at 60^{o}C for 48 hours, ground and then subjected to proximate analysis.

Experiment 2: Determination of the appropriate levels of substituting fishmeal and soybean meal with Moringa leaf meal and housefly maggot meal

This experiment involved determination of the appropriate levels of substituting fishmeal and soybean meal with a mixture of Moringa leaf meal and maggot meal. A feeding trial was carried out to evaluate the effect of substituting fishmeal and soybean meal with a mixture of Moringa leaf meal and housefly maggot meal on growth performance of sex reversed Nile tilapia (*Oreochromis niloticus*). Housefly maggots were produced from wet chicken manure as described in experiment 1 above. Harvesting of maggots was done from day 3 to day 5 after oviposition. Harvested maggots were cleaned, blanched with hot water, oven dried at 60^{o}C for 48hrs and ground into powder using a hammer mill to form maggot meal. Moringa leaves were harvested from *Moringa oleifera* tree orchard at Sokoine University of Agriculture. The harvested leaves were soaked in tap water overnight and then boiled at 80^{o}C for 15 minutes to remove water soluble anti-nutritional factors and deactivate trypsin inhibitor. The boiled leaves were oven dried at 60^{o}C for 48 hrs, and then ground into powder using a hammer mill to form Moringa leaf meal. The processed maggot meal and Moringa leaf meal were mixed together with other ingredients to form tilapia diets and in total, eight diets were formulated as shown in Table 1. Proximate analysis was undertaken for fishmeal, soybean meal, maggot meal, Moringa leaf meal and all formulated diets were analyzed according to AOAC (2000). All diets were formulated to contain about 30% crude protein (Table 2). Diet one (D_1) was a control diet and contained only fishmeal as the source of protein. Diet D_2 was another control diet, but based on soybean plus 5% fishmeal as the sources of protein. For the rest of the diets the percentage of fishmeal was fixed at5%, except diet 3 (D_3) which contained 10% fishmeal. Diets D_3, D_4, D_5, D_6 and D_7 contained various combinations of housefly maggot meal and Moringa leaf meal (Table 1) while diet D_8 contained only housefly maggot meal as sources of protein, addition to 5% fishmeal.

The feeding experiment was conducted using 24 plastic tanks, each with capacity of 30 litre, in an indoor recirculation aquaculture system. Each tank was stocked with 10 Nile tilapia fingerlings with mean initial weight of 1.17 ± 0.04 g. The diets were randomly allocated to the plastic tanks and each diet was replicated three times (i.e., each diet was randomly allocated to three plastic tanks). The fish were manually fed to apparent satiation, but not exceeding 5% of their biomass twice a day at 1000 and 1700 hours. The amount of feed provided was adjusted weekly in accordance with the change in fish's body weight. Water temperature, pH and dissolved oxygen (DO) were measured weekly. Body weight was measured before the start of the experiment and then once per week during the experimental period. The experiment took 56 days. After the experiment the following growth performance parameters were computed: weight gain, growth rate, specific growth rate, feed intake, feed conversion ratio, and protein efficiency ratio.

Table 1: Proportion of different ingredients in the experimental diets

Ingredient	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8
FM (%)	35.00	5.00	10.00	5.00	5.00	5.00	5.00	5.00
HMM (%)	**0.00**	**0.00**	**15.00**	**25.00**	**30.00**	**35.00**	**40.00**	**45.00**
MLM (%)	**0.00**	**0.00**	**50.00**	**45.00**	**30.00**	**20.00**	**10.00**	**0.00**
SBM (%)	0.00	40.00	0.00	0.00	0.00	0.00	0.00	0.00
MM (%)	57.11	44.21	18.92	19.95	30.56	36.00	41.00	46.00
WM (%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
SFO (%)	3.89	6.79	2.08	1.05	0.44	0.00	0.00	0.00
Vit/Min (%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Note: D_1 = control diet 1 with only fishmeal, D_2 = control diet 2 with 40% soybean meal, D_3 = test diet 3 with 15%HMM and 50% MLM, D_4 = test diet 4 with 25%HMM and 45% MLM, D_5 = test diet 5 with 30%HMM and 30% HMM, D_6 = test diet 6 with 35%HMM and 20%MLM, D_7 = test diet 7 with 40%HMM and 10%MLM, D_8 = test diet 8 with 45%HMM and0% MLM.

FM = Fishmeal, HMM = Housefly maggot meal, MLM = Moringa leaf meal, SBM = Soybean meal, MM = Maize meal, WM = Wheat meal, SFO = Sunflower oil, Vit/Min = Vitamin/Mineral mix.

Experiment 3: Determination of the appropriate strategy for minimizing feeding cost

This experiment involved determination of the appropriate level and frequency of feeding farmed Nile tilapia. The best diet identified in study 2 was used in this experiment. The experimental diet was formulated to contain about 30% crude protein, and was prepared using housefly maggot meal (45%) as main protein source, fishmeal (5%), maize meal (46%), wheat flour (2%) and vitamin/mineral premix (2%). The chemical composition of the experimental diet is shown in Table 2. Four feeding strategies were tested. These strategies were considered as treatments for this experiment. The treatments were daily feeding at a level of 2.5% of fish weight (T_1), daily feeding at a level of 5% of fish weight (T_2), feeding one day at the level of 2.5% followed by one day feeding at the level of 5% of fish weight (T_3) and feeding two days at the level of 2.5% followed by two days feeding at the level of 5% of fish weight (T_4). The treatments were randomly allocated to 12 round concrete tanks, each with a size of about 4.5 m^2 and 1 m depth. Each treatment was replicated three times. Nile tilapia (*O. niloticus*) fingerlings with average body weight of 10.34 g were stocked in the concrete tanks at a stocking rate of 10 fingerlings per tank. The fingerlings were randomly allotted to the concrete tanks and the experiment was conducted in a completely randomized design. The fish in each treatment were fed twice per day at 1000 and 1700 hours. Each fish was weighed at the start of the experiment and then on every fourteenth day and the amount of feed offered for each treatment was adjusted accordingly. The experiment was conducted for 70 days. Final body weight of each fish was recorded and weight gain, daily weight gain and specific growth rate were computed. Furthermore, the amount of feed provided was recorded daily and feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) for the whole experimental period were computed. Death of fish was recorded as it occurred and survival rate at the end of the experiment was calculated. Also feed costs, revenue from sale of fish and gross margin were calculated.

Table 2: Chemical Composition of the Diet

Parameter	Percent (%)
Dry matter (DM)	94.81
Crude protein (CP)	32.33
Crude fibre (CF)	9.17
Ether extract (EE)	5.84
Ash	10.00
Gross energy (GE) (kJ/kg)	17.65

Statistical analysis

Data generated on yield and chemical composition of maggots in experiment 1 and on growth performance (final weight, weight gain, growth rate, specific growth rate) and feed utilization parameters (FI, FCR, and PER) in experiment 2 were analysed using GLM procedure of SAS (2003)in a completely randomized design. In experiment 1, an analysis of variance was carried out to assess the effects of substrate, chicken manure quantity, age of maggots at harvest and interaction of chicken manure quantity and maggot age at harvest on maggot yield. In experiment 2, an analysis of variance was conducted to assess the effects of diet on growth performance and feed utilization of Nile tilapia. In experiment 3, an analysis of variance was conducted to assess the effects of feeding level, feeding frequency, and interaction of feeding level and frequency on growth performance of Nile tilapia. In all treatments Tukey's test was used to determine the significance of the differences between a pair of treatment means.

RESULTS

Experiment 1: Determination of the most suitable substrate for maggot production

Five substrates were tested for their suitability as media for production of housefly maggots. The yield of maggots obtained from the different substrates are shown in Table 3. The results show that the type of substrate significantly influenced the yield of maggots ($p \leq 0.05$) (Table 2). The use of cattle offal resulted in significantly higher maggot yield ($p \leq 0.05$) compared to other substrates. The quantity of maggots produced from chicken manure was higher ($p \leq 0.05$) than that obtained from pig manure, cattle manure and kitchen leftovers. The lowest yield of maggots was obtained from cattle manure.

Temperature was significantly higher in chicken manure ($p<0.05$) than in other types of substrates. The temperatures observed in cattle manure, pig manure, cattle offal and kitchen leftovers did not differ significantly and ranged from 32.66 ± 3.50°C in cattle manure to 34.86 ± 4.45°C in kitchen leftovers. The results for correlation analysis indicate that there was a weak relationship between temperature and maggot yield ($r = 0.191$).

Table 3: Maggot yield and temperature from different types of substrates

No	Substrate	Yield (g/kg of substrate)	Temperature (°C)
1	Cattle manure	21.57 ± 0.21^{d}	32.66 ± 3.50^{b}
2	Poultry manure	40.83 ± 0.67^{b}	41.60 ± 3.45^{a}
3	Pig manure	27.25 ± 1.29^{c}	34.66 ± 3.25^{b}
4	Cattle offal	50.47 ± 0.43^{a}	33.46 ± 5.05^{b}
5	Kitchen leftovers	27.24 ± 0.33^{c}	34.86 ± 4.45^{b}

The study carried out to evaluate the effect of quantity of chicken manure on maggot yield indicate that the amount of manure used as substrate significantly influenced ($p \leq 0.001$) the yield of maggots (Table 4). On average the increase of the quantity manure from 6 to 12 kg increased the yield of maggots from 54.49 ± 1.43 to 74.72 ± 1.43 g/kg of manure. Also the results shown in Table 3 indicate that the age of maggots at harvest had significant effect on maggot yield. Maggots harvested five days after hatching showed significantly higher yield (72.23 g/kg of manure) than those harvested four days after hatching (65.27 g/kg of manure), which, in turn, had higher yield than those harvested three days after hatching (56.33 g/kg of manure).

Table 4: Effects of manure quantity and age at harvest on yield of housefly maggots (mean ± se) (g/kg of manure)

	Age of maggots at harvest			
Manure mass	**3-days old**	**4-days old**	**5-days old**	**P-Value**
6 kg	47.47 ± 1.41^{c}	55.12 ± 1.38^{b}	60.87 ± 1.41^{a}	0.0001
12 kg	65.18 ± 1.73^{c}	75.41 ± 1.68^{b}	83.58 ± 1.73^{a}	0.0001

[abc]Means with different superscripts within the same row are significantly different at $p \leq 0.001$.

Table 5 shows the proximate composition of maggots harvested at different ages. On average, maggot meal had crude protein, fat, crude fibre and ash contents of 46.69, 25.92, 6.58 and 9.10%, respectively. The results show that age of maggot at harvest had significant effect ($p \leq 0.05$) on crude protein, ether extract (fat), crude fibre and ash contents. Crude protein content significantly decreased ($p \leq 0.001$) with increase age at harvest of maggot. Fat, crude fibre and ash contents were found to significantly increase ($p \leq 0.05$) with advancement age at harvest of maggot. For increased biomass yield and relatively high protein content it is worth harvesting housefly maggots when they are five days old.

Table 5: Effect of maggot age at harvest on chemical composition of housefly maggot meal

Chemical composition parameter	Age of maggots			P-Value
	3-days	4-days	5-days	
Crude protein (%)	48.67±0.31[a]	46.78±0.31[b]	44.62±0.31[c]	0.0003
Ether extract (%)	23.06±0.21[c]	25.39±0.21[b]	29.30±0.21[a]	0.0001
Crude fibre (%)	6.24±0.01[c]	6.35±0.01[b]	7.15±0.01[a]	0.0001
Ash (%)	8.88±0.01[b]	8.90±0.01[b]	9.52±0.01[a]	0.0001

[abc]Means with different superscripts in the same row are significantly different ($p \leq 0.001$).

Experiment 2: Determination of the appropriate levels of substituting fishmeal and soybean meal with Moringa leaf meal and housefly maggot meal

Table 6 shows the chemical composition of maggot meal, Moringa leaf meal, fishmeal, soybean meal and formulated diets used in the experiment. Soybean had the highest dry matter content, followed by maggot meal. Fishmeal had the highest crude protein content (60.59%), followed by maggot meal (46.96%) while Moringa leaf meal had the lowest crude protein content. Crude fat content was highest in maggot meal (23.12%) and lowest in Moringa leaf meal (6.40%). Maggot meal had the highest crude fibre content while fishmeal had the lowest crude fibre content. The highest ash content was observed in fishmeal while the lowest value was found in soybean meal. The formulated diets had almost the same crude protein contents and ranged from 30.15% in diet D_7 to 31.20% in diet D_2. Diet D_6 (14.88%) and D_8 (14.80%) had the highest fat contents while diet D_1 (9.70%) had the lowest. Diet D_1 had the lowest crude fibre content (2.83%) while diet D_6 had the highest value (7.01%). Ash content was highest in diet D_1 (17.7%) and lowest in diet D_2 (11.23%).

Figure 1 shows the growth performance of Nile tilapia fed different dies during the experimental period. Generally fish fed diet D_1 and D_8 showed the highest growth performance while those on diet D_5 had the lowest growth performance throughout the experimental period. The growth performances of fish fed diets D_2, D_3, D_4, D_6 and D_7 did not differ significantly. The analysis of variance revealed that the type of diet had significant effect ($p \leq 0.05$) on final body weight, weight gain, average daily weight gain and specific growth rate. Fish fed diet D_1 had the highest body weight gain, average weight gain and specific growth rate, followed by those on D_8 and D_6 (Table 7). Fish fed diet D_5 had the lowest body weight gain and average daily weight gain. Table 6 also shows that the type of diet significantly influenced ($p \leq 0.05$) feed utilization efficiency. Fish fed diet D_1 and D_8 had higher feed intake values while those fed diet D_5 had lower value than the rest of the diets. Feed conversion ratio (FCR) was lower on fish fed diet D_1 and D_6, but was higher on those fed diet D_7 and D_4. The type of diet had no significant effect ($p > 0.05$) on protein efficiency ratio. However, diets D_1 and D_3 showed slightly higher protein efficiency ratio compared to the other diets. Results on survival rate are shown in Table 6. The results indicate that survival rate differed significantly among the fish fed different diets. Fish fed diet D_4, D_6, D_7, D_1 and D_8 had significantly higher survival rate compared to those on diet D_2 and D_5.

Table 6: Chemical composition of maggot meal, Moringa leaf meal , fishmeal and formulated diets used in the experiment

	Dry matter (%)	**Crude protein (%)**	**Ether extract (%)**	**Crude fibre (%)**	**Ash (%)**
Maggot meal	94.26	46.96	23.12	10.58	8.51
Moringa leaf meal	93.76	28.60	6.40	9.83	7.14
Fishmeal	93.09	60.59	9.44	0.24	20.74
Soy bean meal	95.12	40.36	14.86	8.42	5.20
D1 (CON 1, FM)	92.46	30.52	9.70	2.83	17.7
D2 (CON 2, SBM)	92.31	31.20	13.32	6.65	11.23
D3 (15%HMM/50%MLM)	92.33	30.83	11.25	6.95	11.72
D4 (25%HMM/45%MLM)	92.68	30.77	12.36	6.75	11.39
D5 (30%HMM/30%MLM)	93.43	30.59	10.32	5.10	12.23
D6 (35%HMM/20%MLM)	92.22	31.13	14.88	7.01	11.93
D7 (40%HMM/10%MLM)	92.60	30.15	11.32	6.83	11.41
D8 (45%HMM/0%MLM)	92.00	30.68	14.80	5.23	11.33

DM = dry matter, FM = fishmeal, SBM = soy bean meal, HMM = housefly maggot meal, MLM = moringa leaf meal, CON 1 = control 1 (FM inclusion), CON 2 = control 2 (SBM inclusion).

Results for water quality parameters, specifically pH, dissolved oxygen (DO) and temperature, during the experimental period are shown in Table 8. Diet had no significant effects on DO and temperature, but significantly influenced water pH. Plastic tanks subject to diet D_4 had higher pH values than those on diet D_6, but the two did not differ significantly from the plastic tanks subjected to the rest of the diets. Generally the values of DO, temperature and pH observed during the whole experimental period were within the range suitable for growth of *O. niloticus*.

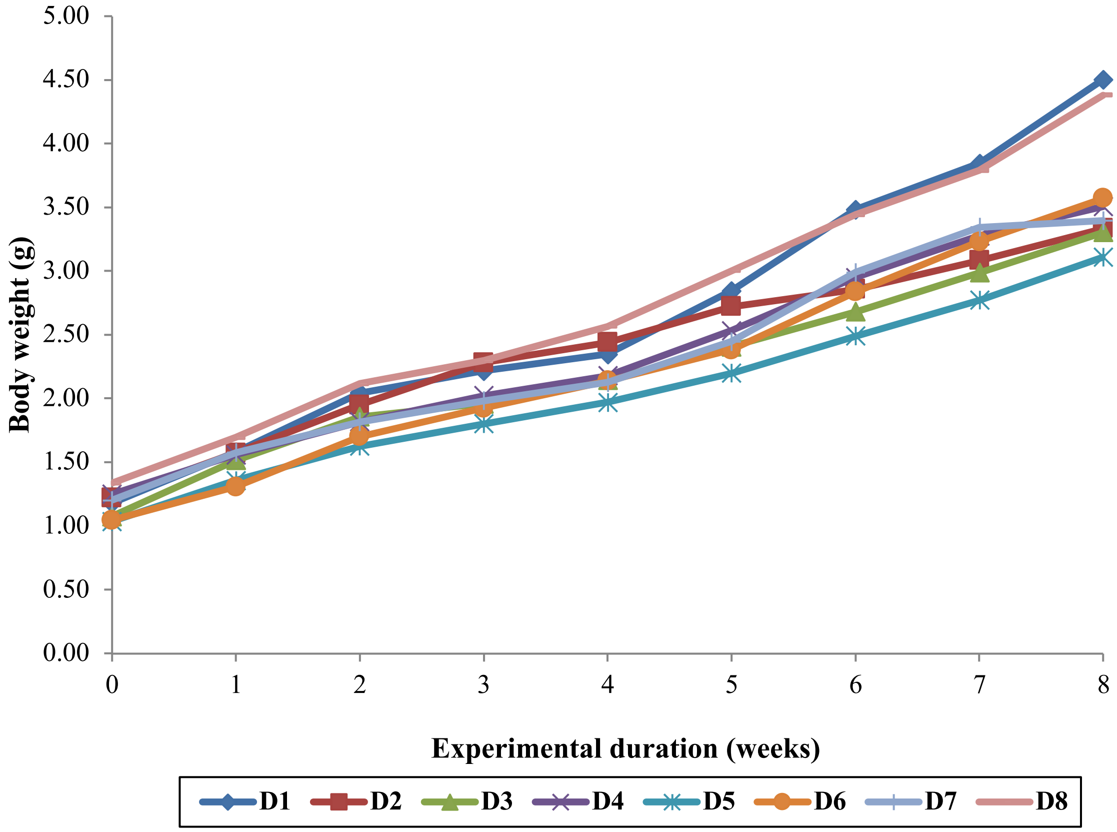

Figure 1: Growth Pattern of Nile Tilapia fed HMM and MLM Diet

Table 6: Growth Performance and Nutrient Utilization of *Oreochromis niloticus* fed diets containing different inclusion levels of housefly maggot meal and Moringa leaf meal

Parameter	D1 (CON 1) 0% HMM, 0%MLM	D2 (CON 2) 5% FM, 40% SBM	D3 15%HMM, 50%MLM	D4 25%HMM, 45%MLM	D5 30%HMM, 30%MLM	D6 35%HMM, 20%MLM	D7 40%HMM, 10%MLM	D8 45%HMM, 0%MLM
IWT (g)	1.18 ± 0.10	1.22 ± 0.09	1.08 ± 0.10	1.25 ± 0.10	1.04 ± 0.09	1.05±0.09	1.20±0.09	1.34±0.10
FWT (g)	4.50 ± 0.23^{a}	3.3.3 ± 0.22^{bc}	3.30 ± 0.22^{bc}	3.51 ± 0.24^{abc}	3.11 ± 0.21^{c}	3.57 ± 0.21^{abc}	3.40 ± 0.22^{bc}	4.38 ± 0.23^{ab}
WTG (g)	3.32 ± 0.25^{a}	2.11 ± 0.23^{b}	2.23 ± 0.24^{ab}	2.26 ± 0.25^{ab}	2.07 ± 0.23^{b}	2.52±0.23^{ab}	2.19 ± 0.23^{ab}	3.04 ± 0.24^{ab}
ADG (g fish^{-1} day^{-1})	0.059 ± 0.002^{a}	0.037 ± 0.002^{bc}	0.046 ± 0.002^{bc}	0.039 ± 0.002^{c}	0.040 ± 0.002^{c}	0.050 ± 0.002^{c}	0.042 ± 0.002^{c}	0.054 ± 0.002^{ab}
SGR (% day^{-1})	2.91 ± 0.104^{a}	2.54 ± 0.104^{abc}	2.81 ± 0.104^{ab}	2.55 ± 0.104^{c}	2.59 ± 0.104^{abc}	2.69 ± 0.104^{abc}	2.45 ± 0.104^{bc}	2.56 ± 0.104^{abc}
FI (g fish^{-1} day^{-1})	0.12 ± 0.003^{ab}	0.11 ± 0.003^{bc}	0.10 ± 0.003^{cd}	0.11 ± 0.003^{bc}	0.09 ± 0.003^{d}	0.10 ± 0.003^{cd}	0.109 ± 0.003^{bcd}	0.127± 0.003^{a}
FCR	2.34 ± 0.06^{c}	2.73 ± 0.06^{bc}	2.55 ± 0.06^{abc}	2.83 ± 0.06^{a}	2.73 ± 0.06^{bc}	2.46 ± 0.06^{bc}	2.84 ± 0.06^{a}	2.56 ± 0.06^{abc}
PER	1.64 ± 0.08^{a}	1.42 ± 0.08^{a}	1.60 ± 0.08^{a}	1.27 ± 0.08^{a}	1.48 ± 0.08^{a}	1.52 ± 0.08^{a}	1.42 ± 0.08^{a}	1.43 ± 0.08^{a}
Survival (%)	98.33 ± 0.96^{a}	92.08 ± 0.96^{c}	95.41 ± 0.96^{abc}	99.16 ± 0.96^{a}	92.91 ± 0.96^{bc}	98.75 ± 0.96^{a}	98.75 ± 0.96^{ab}	97.91 ± 0.96^{a}

^{abc} Means with different superscript letter in the same row are significantly different at $p<0.05$.
IWT = initial weight, FWT = final weight, ADG = average daily gain, SGR = specific growth rate, FI = fed conversion ratio, FCR = food conversion ratio, PER = protein efficiency ratio. Values represent the mean and standard deviation.

Table 4: Water Environment Parameters During the Feeding Experiment

Diet	Parameter		
	pH	**DO (*ppm*)**	**Temp (°C)**
D1 (CON 1, FM)	6.94 ± 0.07^{ab}	6.26 ± 0.05^{a}	24.00 ± 0.01^{a}
D2 (CON 2, SBM)	6.76 ± 0.07^{b}	6.27 ± 0.05^{a}	24.07 ± 0.01^{a}
D3 (15%HMM/50%MLM)	6.83 ± 0.07^{ab}	6.24 ± 0.05^{a}	24.01 ± 0.01^{a}
D4 (25%HMM/45%MLM)	7.15 ± 0.07^{a}	6.32 ± 0.05^{a}	24.07 ± 0.01^{a}
D5 (30%HMM/30%MLM)	6.92 ± 0.07^{ab}	6.26 ± 0.05^{a}	24.05 ± 0.01^{a}
D6 (35%HMM/20%MLM)	6.75 ± 0.07^{b}	6.16 ± 0.05^{a}	24.03 ± 0.01^{a}
D7 (40%HMM/10%MLM)	6.83 ± 0.07^{ab}	6.31 ± 0.05^{a}	24.07 ± 0.01^{a}
D8 (45%HMM/0%MLM)	6.82 ± 0.07^{ab}	6.11 ± 0.05^{a}	24.02 ± 0.01^{a}

^{abc} Means with different superscript letter in the same column are significantly different at $p<0.05$.
DO = dissolved oxygen, Temp = temperature, FM = fishmeal, SBM soybean meal, CON1 = control 1, CON 2 = control 2.

Experiment 3 - Determination of the appropriate strategy for minimizing feeding cost

Figure 2 shows the growth performance of Nile tilapia reared under different treatments. Fish under treatment T_3 showed higher ($p \leq 0.05$) growth performance than those on T_1, T_2 and T_4. The differences in fish body weight between the fish under T_3 and those under T_1, T_2 and T_4 were obvious just from the second week of the experiment. The results in Table 9 show that the mean final body weight, weight gain, average daily gain and specific growth rate of fish reared under T_3 were significantly higher than of those under T_1, T_2 and T_4. But fish under T_1, T_2 and T_4 did not differ significantly in terms of mean final body weight, weight gain, average daily gain and specific growth rate, though those under T_1 had the lowest values. The highest feed intake was observed on fish under T_2, followed by those under T_3; while those under T_1 had the lowest feed intake. Feed conversion ratio (FCR) of fish under T_1 was significantly lower compared to that of those under T_2, T_3 and T_4. On the other hand, the fish reared under T_2 had significantly higher FCR than those under T_3 and T_4. Fish under T_1 had significantly higher protein efficiency ratio (PER) while those under T_2 had lower PER than those under T_3 and T_4. The survival rate did not differ significantly among the fish reared under different treatments, though the fish under T_2 showed the highest survival rate while those under T_1 had the lowest. The mean ± s.e. values for water quality parameters were: pH = 7.63±0.1, dissolved oxygen (DO) = 7.03±0.3 mg/l and temperature = 26.21±0.32°C.

Table 10 shows estimated yield, feed cost, fingerling cost, labour cost, total variable cost, revenue and gross margin obtained from the fish cultured under different feeding levels and frequencies. Fish cultured under T_3 showed the highest fish yield, revenue and gross margin while those under T_1 showed the lowest fish yield and revenue. The highest feed cost and total variable cost were observed on fish under T_2 while the lowest were found on T_1.

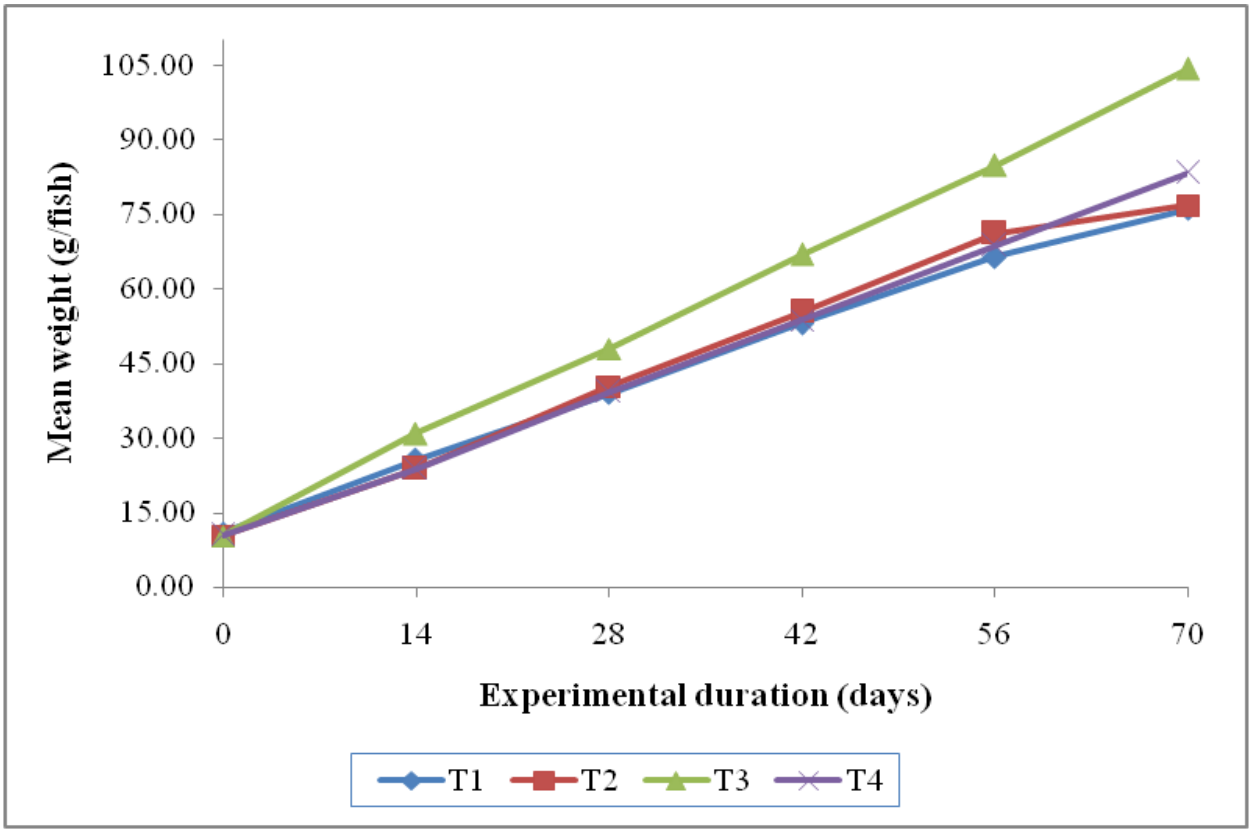

Figure 2: Growth performance of *O. niloticus* subjected to different feeding levels and frequencies

Table 9: Effect of feeding level and frequency on growth performance, feed utilization and survival of *O. niloticus*

	Treatments					
Parameter	T_1	T_2	T_3	T_4	SEM	*P-value*
Initial weight (g)	10.80	10.20	10.29	10.41		-
Final weight (g)	75.96^b	76.72^b	104.22^a	83.33^b	3.48	0.0038
Weight gain (g)	65.17^b	66.52^b	93.92^a	72.91^b	3.46	0.0036
Average daily gain (g/day)	1.22^b	1.26^b	1.60^a	1.30^b	0.07	0.0036
Specific growth rate (%/day)	4.22^b	4.39^b	5.13^a	4.31^b	0.13	<0.0001
Feed intake (g/day)	0.90^c	1.86^a	1.67^a	1.36^b	0.06	<0.0001
Feed conversion ratio	0.86^c	1.68^a	1.15^b	1.23^b	0.04	<0.0001
Protein efficiency ratio	0.84^a	0.41^c	0.67^b	0.58^b	0.05	<0.0001
Survival rate (%)	86.66^a	96.66^a	93.33^a	90.00^a	5.77	0.6588

^{abc} Means with different superscript letter within the same row are significantly different at $p \leq 0.05$.

Table 10: Comparison of feed cost, revenue and gross margin obtained from fish cultured under different feeding levels and frequencies

Variable	Treatment (mean)			
	T_1	T_2	T_3	T_4
Estimated yield (kg/ha/year)	8,777.6	9,888.4	12,969.6	9,999.60.6
Revenue (TZS)	78,998,400.0	88,995,200.0	116,726,400.0	89,996,400.0
Fingerlings costs (TZS)	11,111,111.1	11,111,111.1	11,111,111.1	11,111,111.1
Feed cost (TZS)	11,469,920.0	25,974,666.7	22,635,262.2	17,400,631.1
Labour cost (TZS)	4,500,000.0	4,500,000.0	4,500,000.0	4,500,000.0
Total variable cost (TZS)	27,081,031.1	41,585,777.8	38,246,373.3	33,011,742.22
Gross margin (TZS)	51,917,368.89	47,409,422.2	78,480,026.67	56,984,657.8

DISCUSSION

Determination of the most suitable substrate for maggot production
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Maggot, the larvae of the domestic fly (*Musca domestica*) has ability to grow on a large range of substrates. This study assessed the suitability of five substrates for production of maggots. The results indicate that the highest yield of maggots was obtained from cattle offal, followed by chicken manure. Despite the fact that cattle offal produced higher quantity of maggots than chicken manure, chicken manures seem to be the most suitable substrate for culturing housefly maggots because they are readily available and can be obtained at no cost. The findings in this study agree with Odesanya *et al.* (2011) and Ezewudo *et al.* (2015) who reported that chicken droppings are the most suitable substrate for production of housefly maggots. In the present study the use of chicken manure as attractants for houseflies and substrate for culturing the resulting maggots produced significantly large quantity of maggots, almost double, compared to pig manure, cattle manure and kitchen leftovers. This may be due to higher content of nitrogen in chicken manure compared to cattle and pig manure. The observation in the present study is in agreement with Obeng *et al.* (2015) who reported that poultry waste is a better substrate for production of maggots.

Determination of the appropriate levels of substituting fishmeal and soybean meal with Moringa leaf meal and housefly maggot meal

Maggot meal has been reported to be a promising alternative to the expensive protein sources as it has good nutritional value and cheaper compared to other animal protein sources. The results in this study show that maggot meal had higher crude protein content than soybean meal and Moringa leaf meal, but lower than that of fish meal. This shows that maggot meal is better source of protein than soybean and Moringa leaf meals. The CP content of maggot meal observed in this study is almost similar to the CP values of 47.1 and 48.0% reported by Aniebo *et al.* (2008) and Odesanya *et al.* (2011), respectively, but lower than the CP value of 64.0% reported by Hwangbo *et al.* (2009). The high CP content implies that maggot meal has high nutritive value and can provide the amount of protein required by Nile tilapia for proper growth. The values crude fibre, fat and ash contents obtained in this study are close to that reported by Odesanya *et al.* (2011) and Aniebo *et al.* (2008).

The results of a feeding trial show that fish fed the diet based on maggot meal (D_8) as the main source of protein had body weight gain, growth rate and FCR that were not significantly different from that of fish fed diet based on fishmeal as the source of protein (D_1). This observation is in agreement with the findings of Ogunji *et al.* (2006), who suggested that maggot meal can completely replace fishmeal in the diet of Nile Tilapia (*Oreochromis niloticus*) and can meet the nutrient requirements of this species. Makinde (2015) recommended that the inclusion of maggot meal in fish diets should be limited to 25-30% as performance tends to decrease when higher inclusion levels are used. In the present study the percentage of maggot meal was increased from 0, 15, 25, 30, 35, 40 to 45% of the diet and growth performance and FCR were better at the inclusion level of 45%, almost comparable to the control diet based on fish meal. At inclusion level of 45%, fishmeal was replaced with maggot meal in diet D_8 by about 86% and the growth performance and survival did not differ significantly from that of the diet containing 100% fishmeal. This demonstrates that maggot meal is an ideal protein source and can completely substitute fishmeal. Sogbesan *et al*. (2006) evaluating the use of maggot meal in the diet of *Heterobranchus longifilis* x *Clarias gariepinus* hybrids concluded that 100% replacement of fishmeal with maggot meal is an economically viable option.

Soybean has been identified as most attractive plant protein source, palatable to most fish species and can successfully replace fishmeal up to 75% (El-Sayed, 1999). When comparison is made between maggot meal and soybean meal in the current study, fish fed the diet based on maggot meal (D_8) as the source of protein had higher growth performance and better FCR than those fed the soybean meal based diet (D_2). Maggot meal had higher crude protein content than soybean meal, thus fish fed diets based on maggot meal showed faster growth. This indicates that maggot meal is a better source of protein than soybean meal and can be used to replace fishmeal in fish diets.
The present study evaluated the effects of including different combination levels of Moringa leaf meal and maggot meal in Nile tilapia diets. Despite its high nutritional quality, inclusion of Moringa leaf meal in the diet significantly reduced the growth performance and feed utilization. The lower growth performance of the fish fed diets containing Moringa leaf meal may due to the relatively low protein content as the CP content of Moringa leaf meal was significantly lower than that of fishmeal, soybean meal and maggot meal. This could be indicate ineffectiveness of method used to remove inherent anti-nutritional factors namely tannins, trypsin and amylase inhibitors, lectins, cyanogenic glucosides, saponins, phenols and phytic acid. These are known to reduce palatability, intake and nutrient bioavailability from Moringa leaf meal (Makkar and Becker, 1996; Makkar and Becker, 1997; Afuang *et al*., 2003).Richter *et al*. (2003) showed that saponins and tannins can reduce growth in *Oreochromis niloticus*. Thus, there is a need to devise more effective methods of removing inherent anti-nutritional factors to enable its use as a protein source in tilapia diets.

Determination of the appropriate strategy for minimizing feeding cost
Feed is the highest variable cost in aquaculture enterprise. Therefore, it is important to establish a strategy for using appropriate amount of feed in order to reduce waste and increase profit (Ali *et al*., 2016). This study compared the growth performance, feed cost and gross of margin of fish reared under the treatments of daily feeding at 2.5% of fish weight (T_1), daily feeding at 5% of fish weight (T_2), one day alternating feeding at 2.5 and 5% of fish weight (T_3), two days alternating feeding at 2.5 and 5% of fish weight. The results indicate that fish cultured under T_3 showed better growth, feed utilization and gross margin compared to fish under other treatments. Thus alternating the feeding levels of 2.5 and 5% of fish body weight in consecutive days is the appropriate feeding strategy for minimizing cost. Feeding level of 5% of body weight daily was probably excessive and resulted in most of the feed being left uneaten. This uneaten feed decayed and polluted the water and thus retarded growth. On the other hand, daily feeding at 2.5% of the body weight resulted into inadequate feeding. It appears that at the feeding level of 2.5% of body weight, a large proportion of nutrient in the diet was used to meet maintenance requirements, and only a small proportion was available for growth and this retarded growth of the fish. The relatively lower (better) FCR obtained on fish under T_3 confirms that alternating the feeding levels of 2.5 and 5% of fish body weight for one day is the best feeding strategy. This agrees with Dwyer *et al*. (2002) who said that feed efficiency and growth are decreased when fish are either inadequately or over fed. The findings of the current study contradict the findings of Deyab and Hussein (2015) who suggested that feeding rate of 5% of body weight daily significantly enhances fish growth and feed utilization and can be considered as the optimal feeding rate for red tilapia fingerlings. Also the findings of this study disagrees with the findings of Abdelghany and Ahmad (2002) who reported that feeding to apparent satiation (feed amount of equivalent to 2.67% of fish body weight per day) supports higher fish production, income and net profit and hence, it is the appropriate optimal feeding level.

Although feed cost was significantly lower for the fish cultured under T_1, gross margin was higher in fish under daily alternate feeding of 2.5 and 5% of body weight than those fed daily on either 2.5 or 5% of body weight. According to Bolivar and Jimenez (2006) alternate day feeding reduces production costs of tilapia without significantly affecting performance as it uses almost 50% less feed compared to fixed daily feed ration. In the present study, alternating the feeding rate between 2.5 and 5% of body weight every two days, though lowered feed cost, it negatively affected growth and subsequently yield at harvest. This led to lower revenue and profit than alternating daily feeding of the two levels. Therefore, this study has found that daily alternating feeding levels of 2.5 and 5% of body weight daily is a better feeding strategy for cost minimization and profit maximization.

CONCLUSIONS

Generally, fish fed fishmeal based diet (D_1) showed the highest growth performance, followed by those fed housefly maggot meal based diet (D_8) while those fed the diet containing 30% housefly maggot meal and 30% Moringa leaf meal had poor performance. The study has demonstrated that housefly maggot meal alone can replace fish meal as the source of protein in the diets for Nile tilapia. The lack of significant difference in protein efficiency ratio values between the diets containing fishmeal and maggot meal implies that maggot meal has biological value almost equivalent to fishmeal. Furthermore, this study has demonstrated that alternating feeding levels of 2.5 and 5% of fish weight (T_3) daily is the best feeding strategy and can be used to increase the profitability of aquaculture enterprise. Based on the results from this study, the following conclusions can be made:-

i. Chicken manure is a better substrate than both cattle manure and pig manure for production of housefly maggots.
ii. Increasing the quantity of chicken manure in the facilities for production of maggots can increase significantly the yield of maggots.
iii. Housefly maggot meal has higher protein content and promotes better growth performance than soybean meal.
iv. Housefly maggot meal alone can be used to replace fish meal in the diets without affecting the growth performance of Nile tilapia.
v. Mixture of housefly maggot meal and Moringa leaf meal gives better growth performance compared to soybean meal, hence, can be used in tilapia diets instead of soybean meal.
vi. Alternating feeding levels of 2.5 and 5% of body weight on consecutive days is the most appropriate feeding strategy for Nile tilapia compared to continuous daily feeding at either 2.5 or 5% of body weight.

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ENHANCING THE NUTRITIONAL VALUE OF TILAPIA FOR HUMAN HEALTH

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT02PU

Rebecca Lochmann[1], Nelson W. Agbo[2], and Regina Edziyie[2]

[1]*University of Arkansas at Pine Bluff, Pine Bluff, AR USA*
[2]*Department of Fisheries & Watershed Management Kwame Nkrumah University of Science & Technology, Kumasi, Ghana.*

ABSTRACT

Fish have traditionally been regarded as an important and wholesome source of nutrition for humans. Ghana's fish intake is known to exceed that of the world average. Cultured fish are known to have higher levels of n-6 than n-3 fatty acids and this is directly linked with the quality of the ingredients used to prepare feeds for growing such fish. Therefore, by altering the diets of cultured fish to have higher n-3 fatty acids it is likely to enhance the nutritional value of the fish grown and increase the benefits derived by consumers. This study focused on identifying local sources of feed ingredients high in n-3 fatty acids, determining the impact of diets high in n-3 fatty acids on the flesh quality of fish, and benefits to consumers. To determine sources of feed ingredients rich in n-3 fatty acids, a survey was conducted with targeting oilseed sellers and processors. Additionally, secondary data on oilseeds were obtained from three leading research institutions and from general literature search because there was no database that had such information. Fatty acid composition of a select group of eight seeds was determined. An 8-week growth study was conducted in $1m^3$ hapas using n-3-rich oils from oilseeds. In all six of the diets that were tested: five were formulated using three different oils and the sixth was a commercial control. The diets were randomly assigned to hapas stocked with Nile tilapia *Oreochromis niloticus* fingerlings of size 28±4.0 g. Fatty acid composition of test diets, control fish and experimental fish carcass were quantified. Although, the select ingredients had high levels of lipids, none of them had appreciable levels of n-3 fatty acids (> 1%).

INTRODUCTION

Both n-3 and n-6 fatty acids are essential nutrients for humans, but there is a striking imbalance in the intake of n-3 and n-6 fatty acids globally. Intake of n-6 fats far exceeds that of n-3 fats due to the widespread consumption of plant oils and grains that contain more n-6 than n-3 fatty acids (Trushenski and Lochmann 2009). Thus, traditional diets can lead to marginal to severe deficiencies of n-3 fatty acids and a variety of associated health problems, such as cardiovascular disease, arthritis, atherosclerosis, diabetes, and cancer (Horrocks and Yeo, 1999; Arterburn et al. 2006; Simopoulos, 2008). In infants, deficiency of n-3 fatty acids can be especially destructive, as fatty acids such as DHA are crucial for normal brain development, behaviour, and cognitive function (Innis, 2007).

In Ghana, n-3 deficiency is more common in infants and pregnant or lactating women than in adult males (Siekmann and Huffman, 2011). Currently, the recommended n-3 range is from 500-1500 mg/day depending on factors such as age, gender, reproductive status, and prior history of coronary problems.

Fish are the primary practical source of n-3 fatty acids in most countries (Tocher, 2003), and fish supplies approximately 60% of the protein for the population of Ghana. It is well known that fish acquire the fatty acid signatures of their diets (they "are what they eat") (Turchini et al. 2011). Farmed tilapia have been criticized for being too high in n-6 and too low in n-3 fatty acids (Weaver et al. 2008). Fish feed typically contain large amounts of plant ingredients high in n-6 fatty acids. Although, these ingredients are considered environmentally sustainable to use in fish diets, they lack the healthy n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) found in marine fish products. With the global and local decline in fish catches it would be preferable to identify viable plant sources of n-3 fatty acids for long-term growth and sustainability of the aquaculture industry in Ghana. Fortunately, tilapia can elongate and desaturate 18:3n-3 found in plant oils to form n-3 LC-PUFAs such as 20:5n-3 and 22:6n-3. Therefore, inclusion of preformed LC-PUFAs is not necessary for the general performance or health of the fish (NRC, 2011).

The suitability of feed ingredients for commercial production of both fish and feed are based not only on nutrient content, but on economics and availability, as well as palatability to the fish (Hardy and Barrows, 2002). In Ghana, a large number of oilseed and cereal by-products are available for screening as potential sources of n-3 fatty acids for fish feeds (Nelson and Wallace, 1998; Hecht, 2007). A few plants found in Ghana such as *Leucaena leucocephala* and *Moringa oleifera* contain more than 30% 18:3n-3 in their lipids. There is some information on the feeding value of *Leucaena* and *Moringa* in Nile tilapia (Adeparusi and Agbede, 2005; Madalla, 2008), but more information is needed to optimize the inclusion levels of these leaf meals in diets to obtain both profitable production and improved product quality (i.e., enhanced content of n-3 fatty acids). A recent study at the University of Arkansas at Pine Bluff (Kasiga, 2012) showed that leaf meals made from these plants could be substituted for up to 30% of the protein in soybean meal in diets of Nile tilapia without reducing fish performance. The leaf-meal diets also significantly increased the concentration of total n-3 fatty acids and n-3 LC-PUFAS in the fish. In addition to the leaf meals, a survey should be conducted to identify any other potential feed ingredients available in Ghana that would enhance the n-3 fatty acid content of the fish and meet the other criteria discussed previously.

Aside from whole plant ingredients, it is possible to add isolated lipid sources to the diets also, such as flaxseed oil. This oil contains more than 50% 18:3n-3, and does not inhibit tilapia growth (Karapanagiotidis et al. 2007). Linseed oil (53% 18:3n-3) is another available source of n-3 fatty acids in Ghana. It is also possible to maximize the retention of the desirable n-3 LC-PUFAs in fish by supplying most of their dietary fat as saturated fat such as coconut oil or palm kernel oil (Trushenski et al. 2009). These oils are widely available in tropical regions and could be used in combination with an n-3 lipid like linseed oil to optimize the n-3 content of the fish, while keeping diet cost as low as possible.

In this study, 20% fish meal is used in diets. The strategy has not been tested in tilapia using diets without fish meal and oil, so further verification of the strategy using diets without marine products is needed. The degree of enrichment and the time needed to achieve a target level of enrichment will depend on the amount of n-3 fatty acids in the diet, the feeding rate, and length of time the fish are fed the diets. The diets will also need to support optimal growth, have high feed conversion rates and survival of the tilapia to minimize cost-of-gain and support production profitability.

The ability to produce tilapia enriched with n-3 acids as a functional food could be a key factor in mitigating widespread health problems associated with essential fatty acid deficiencies in Ghana and other developing countries. The challenge is to identify n-3 sources that will support fish performance and enhance the quality of farmed tilapia for human health while maintaining production profitability. In this project, we sought to identify and nutritionally characterize locally available, cost-effective ingredients that will add n-3 fatty acids to the diets of tilapia, and create an n-3 enhanced product that has greater potential to enhance the health of consumers.

OBJECTIVES

1. Assess the availability and distribution of potential fish feed ingredients containing n-3 fatty acids for use in Nile tilapia diets in Ghana.

2. Determine the proximate composition and fatty acid composition of potential feed ingredients, and recommend specific inclusion rates for diets to be tested in feeding trials with Nile tilapia in Ghana.
3. Analyze the proximate and fatty acid composition of experimental diets and fish from feeding trials in Ghana and determine cost-of-gain of the different diets.
4. Determine the amount of tilapia that would need to be consumed by humans to obtain the target amount of n-3 fatty acids (500-1500 mg/day) for health benefits.

Null hypothesis: There will be no difference in n-3 fatty acid content among fish fed diets with different ingredients.

METHODS AND MATERIALS

Study 1 – Identification and Characterization of Local Sources of Potential Ingredients with High Omega-3 Content

The initial plan was to do a desk study to identify these ingredients, but with the few published studies in Ghana and no database available on possible sources, the search was expanded to include a search of documents in Ministry of Food and Agriculture in Ghana, research institutes and university libraries.

Data collection

In addition to the secondary data acquired from the government and research institutions, primary data on oilseeds were obtained from oilseed sellers and processors (nine) by means of a survey. The survey instrument was a semi-structured questionnaire. The investigation focused on the major crop production and sale areas in Ghana; Brong-Ahafo, Ashanti, Greater Accra, and Upper East regions (5 of 10 administrative regions) of the country.

Proximate, fatty and amino acid determination

Eight of the potential ingredients were selected for further investigations because they were the least researched ones (Table 1). Proximate composition of the ingredients was determined following methods described in AOAC (2005). Lipid extracts from the ingredients were used for the fatty acid analysis. Ten mL of the lipid extracts were evaporated under nitrogen and then trans-esterified with 14% boron trifluoride. The resulting fatty acid methyl esters (FAMEs) was analyzed (Morrison and Smith 1964) using a flame ionization gas chromatograph with helium as the carrier gas. The FAMEs were separated on a fused silica capillary column (15 m x 0.25 mm internal diameter). The 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. The FAMEs were identified and quantified by comparing the retention time and peak area to those of serially diluted mixtures of reference standards. Amino acid analysis was conducted at the University of Missouri.

Study 2 - Growth trial; growth performance of fish fed n-3 enhanced diets and nutritional composition of the fish fed these diets

As there were no appreciable levels of omega-3 fatty acids in the ingredients selected in Study 1, locally available oils (linseed, palm, and soy) were used to replace fish oil in the diets for Study 2. Five diets with different inclusion levels of the selected oils were formulated and compared with a commercial control.

Experimental Design

The growth study was conducted at the Faculty of Renewable Natural Resources farm, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. In all, six diets were tested including a commercial control, each with four replicates. The experimental units were 1 m x 1 m x 1 m hapas, which were mounted in two ponds (blocks) of size 30 m x 10 m each; two ponds were used to prevent overcrowding. The diets were randomly assigned to the hapas in the ponds and the variables that were monitored for growth were food conversion ratio (FCR), specific growth rate (SGR), and weight gain percentage. To determine the effects of the diet on the fatty acid composition of the Nile tilapia, the fatty acid profile of the diets and fish fed the different test diets were also determined.

Test Diets
Five test diets were formulated (Table 5) with the plant oils; linseed oil, soybean oil, and palm oil were used as lipid sources in the feed and a commercial floating feed was used as the control diet. The Winfeed 2.8 feed formulation software was used in formulating the diets. For each feed, the appropriate ingredients were weighed using an electronic scale (Ohaus Navigator XL model NVL2101/1) and these were well mixed in a Kenwood KVC5000 standmixer (Plate 3.2 B) for about 10 minutes. Then 100 mL of water was added to the mixture for easy pelletability and re-mixed in the mixer. The resulting mixture was then passed through a Bosch meat mincer (Propower MFW67440) fitted with 2.0 mm die size; then oven dried at 60°C for 12 hours. The strands of pellets produced were further broken into smaller pieces that could be eaten by the juvenile fish.

Pond preparation
Ponds used for the experiments were completely drained and dried after which the ponds were limed at a rate of 1 kg agriculture lime per 10 m^2. Ponds were then filled with water from a borehole and water levels were maintained above 1 m throughout the experiment. Ponds were fertilized with Mono Ammonium Phosphate (MAP) at 2 g per m^2 and Urea at 3 g per m^2.

Stocking and Feeding
Each 1 m^3 hapa was stocked with 25 all male Nile tilapia fingerlings of average weight of 28±4.0 g procured from the Pilot Aquaculture Centre, Tano-Odumasi and fed rations corresponding to 3% body weight per day of their respective experimental diet randomly assigned them. Feeding was done twice daily at 09:00 am and 4:00 pm.

Fish and Water Sampling
Fish were sampled every two weeks. At each sampling the bulk weight of all the fish in each hapa were taken. The following water quality variables were monitored throughout the study to ensure that water quality remained well within limits recommended for Nile tilapia culture. Water temperature (ºC), pH, and dissolved oxygen (mg/L) were measured twice a week using Hanna (HI 9828) multi-parameter probe. Total ammonia nitrogen and nitrate levels in the pond were determined using the Indophenol and photometric method respectively with a Wagtech photometer whereas nitrate levels were determined using the diazonium method.

Nutritional analyses
The nutritional composition of the ingredients, test diets, control fish, and experimental fish at the end of the study were determined following methods described above.

Cost to gain
Only the lipid sources varied between diets (the control diet was not included in this calculation due to the unknown composition). Since the total cost of the experimental diets were unknown, relative cost-of-gain was determined based only on the cost of lipids in relation to the FCR obtained on each diet.

Statistical Analyses
Data on growth were analyzed using a two-way ANOVA test at an alpha of 0.05, to test for differences due to the treatment (diets) and block effect and a Bonferroni posttest for post hoc comparison. All data were expressed as means ± SD, all graphs and analyses were executed using the Graphpad Prism 5.01 for windows statistical software.

RESULTS

Study 1 – Identification and Characterization of Local Sources of Potential Ingredients with High Omega-3 Content
In all, 23 ingredients locally produced in Ghana were identified based on the survey that was conducted and secondary data sources. Most of these were abundant for short periods (1-2 months) in the year with the exception of Sheanut and palm nut seed/oil, which were abundant for four months of the year.

Of the eight ingredients selected for further investigations, two of them; *Cucumeropsis mannii* (Egusi) and *Citrillus lanatus var neri* (Neri) were common to all the five regions sampled in the study, but the other six potential ingredients were found only in the Brong Ahafo and Upper East Regions. Unfortunately, none of the ingredients contained significant amounts of n-3 fatty acids (<0.5%; Table 2). Hence, a supplemental lipid, linseed oil, was added to the diets to enrich the fish and their human consumers with n-3 fatty acids. Protein content of the ingredients was high, ranging from 21-39% (Table 3). This compares favourably with other plant products such as distiller's grains, but is lower than others such as soybean meal.

Study 2 - Growth trial; growth performance of fish fed n-3 enhanced diets and nutritional composition of the fish fed these diets
Survival was high for the study ranging from 98-100%; there were no significant differences in survival rates for the different diets. Generally, there was a steady growth at almost equal rate at the beginning of the trial for all diets except for the diet with 100% Linseed oil for the first 12 days. After day 12, the control performed better than the experimental diets and among the experimental diets, the one with 100% linseed oil had the poorest growth rate. A similar trend was observed with the FCR and SGR growth parameters.

At the end of the 7-week growth trial, the control diet had the highest weight gain of 92.4±2.0%. The least performing diets in this study were the 100%Lin and the PalmSoy diets with a percentage weight gain of 66.2±2% and 69.2±1% respectively. The rest of the diets had between 70-80% weight gain (Table 6). There were significant differences in weight gain ($p = 0.0012$) between the diets with the differences occurring between the control diet and experimental diets. However, no significant differences were found between the two experimental blocks in the study ($p = 0.859$). The highest SGR was observed in the control diet with 1.32% and the least was 1.02%. in the 100%Lin diet. There were significant differences in SGR ($p = 0.002$) between the control and all the diets except the LinSoy diet. The diet with the best FCR was the control diet with 1.2±0.1 and the least was the LinPalm and 100%Lin diets with 1.62±0.2 and 1.60±0.2, respectively (Table 6). Again, there were significant differences in FCR between the control and all the diets. However, there were no significant differences among the two experimental blocks for both SGR and FCR.

A total of 23 fatty acids were identified in the fish tissues at the end of the experiment. The fatty acids of interest in this study were PUFAs belonging to the omega-3 and omega-6 series. The n-3 content in the fish at the end of the study ranged from as low as approximately 5% in the control diet to a high level of 22.4% in 100% Lin (Figure 2). The diets containing Linseed oil (100% Lin), had high levels of alpha-linolenic acid (n-3) in the fish tissues as compared to the other experimental diets. For the omega-6 fatty acids (n-6), the content in the fish tissues ranged from a low of 17.8% in control diet to the highest level of 31.5% in 100% Soy. Whereas n-3 fatty acids increased considerably in all diets, with reference to the initial sample, n-6 fatty acid showed a decrease. Long chain derivatives of n-3 fatty acids, that is, EPA and DHA fatty acid of fish fed diets with linseed oil, that is 100% Lin, LinSoy and PalmSoy showed slight increase (2.5-3.9%) when compared to levels in the fish prior to the experiment (2.1%). This was however, not statistically significant ($p < 0.05$). There was a significant difference in n-3 in fish flesh between 100% Lin and all the other diets, as well as the initial fish stock. Significant differences in n-3 also occurred between control diet & 100% Soy and between LinSoy & LinPalm.

Cost of gain:
For the experimental diets the relative cost of gain was in the following order:

100% Lin > LinPalm > 100% Soy > LinSoy > PalmSoy

The diet with 50% palm and 50% soybean oil had the lowest relative cost of gain, whereas that with 100% linseed oil had the highest relative cost of gain. The high cost of linseed oil in combination with the high FCR of fish fed that diet explained the latter result.

However, fish fed the diet with 100% linseed oil had the highest total level of n-3 fatty acids. Fish fed diets with LinSoy or LinPalm had intermediate levels of n-3 fatty acids, whereas those fed 100% Soy or PalmSoy oils had the lowest levels of total n-3 fatty acids (Figure 2). Although, the fish in this study were sub-market size, consumption of about 50 grams of fish fed the 100% Lin diet or the LinSoy or LinPalm diets would provide 500 mg or more of total n-3 fatty acids (the lower end of the recommended level). Consumers would need to eat about 5 times more (250 g) of fish fed the 100% Soy or PalmSoy diets to get the same amount of total n-3 fatty acids.

Although, specific levels of the long-chain n-3 fatty acids (mainly EPA and DHA) were not reported for fish fed each diet, a range of 25 – 39 mg could be calculated. The recommended n-3 fatty acid intake (500–1500 mg/day) is actually for the long-chain n-3 fatty acids. The concentration of long-chain n-3 fatty acids was more similar in fish fed the different diets than the total n-3 fatty acids (dominated by alpha-linolenic acid). Therefore, it is estimated that consumption of 250 g of fish fed any of the diets would meet or exceed the minimum recommended level of 500 mg of long-chain n-3 fatty acids. It must be noted that all diets contained 20% fish meal, which would have provided a basal level of about 2% fish oil in all diets. If the level of fish meal inclusion was reduced (to lower diet cost and improve environmental sustainability of tilapia culture), the results of this study might be very different. The effects of different lipids (differing in fatty acid composition) will vary with the overall diet composition, and would be expected to differ when using diets with little or no fish meal compared to diets with 20% fish meal.

DISCUSSION

The upper East and Brong Ahafo regions are important sources of oilseeds in Ghana. The eight oilseeds selected were high in lipid and protein content, but were not considered as important sources of n-3 fatty acids. They could be partially used to replace more expensive protein sources in the diet of tilapia. The good growth performance in fish fed diets with equal proportions of linseed and palm oils could possibly be due to relatively equal proportions of both n-3 and n-6 PUFA supplied by the linseed and soy oil respectively, and explain why the fish fed diet with 100% soybean oil, which was low n-6, had poor growth performance. According to Takeuchi *et al.* (1983) and Aksoy *et al.* (2009), tilapia has a higher requirement of n-6 to n-3 PUFA. The poor growth rate in the linseed only diet could be observed right after the 1st sampling and continued though the end of the study, whereas, the differences in the rest of the diets were only prominent within the last 12 days of the experiment. This study showed similar results compared to the findings of Chen *et al.* (2013); the muscle fatty acid composition of Nile tilapia juveniles fed diets with n-3 fatty acids was reflective of the content in the diet. They determined that the optimum requirement of Nile tilapia juveniles was 0.45-0.64% of dry weight.

CONCLUSIONS

Twenty oilseeds were identified in five of the ten regions in Ghana. The Brong Ahafo and Upper East region had the most number of oilseeds. With the Egusi and Neri seeds that were available for four months in a year, the others were available for 1-2 months within the year. The oil seed/ingredients had low n-3 levels (< 0.5%), but high levels n-6 fatty acids; the most abundant fatty acids in all the seed were the linoleic and oleic acids. Protein content was between 21-39%.

The n-3 concentration in the fish at the end of the study were highly reflective of the composition their diet; ranging from 3.5% (control) to 22% (diet with 100% Linseed oil) compared to 17.8% (control) to 31.5% (100% soybean oils diet). The diet with 100% linseed oil had significantly higher levels of n-3 than the rest, however, the high n-3 content seem to suppress the growth of the juvenile tilapia and had the highest relative cost to gain. There were similar trends in the weight gain, feed conversion ration and specific growth rate (SGR) with the control diet generally performing better than the test diets. An exception was the 50:50 linseed and soybean oil diet which had comparable SGR with that of the control. Based on our findings, a person would have to consume 250 g of fish fed any of the test diets to get the minimum recommended level of long chain n-3 fatty acids.

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TABLES AND FIGURES

Table 1. Ingredients from Ghana analyzed for nutrient content to assess potential for tilapia diets

Calabash seeds	(*Lagenaria siceraria*)
Eggplant seeds	(*Cucumis spp.*)
Sesame seeds	(*Sesamum indicum*)
Kidney beans	(*Phaseolus lunatus*)
Neri seeds	(*Citrillus lanatus*)
Egushi seeds	(*Cucumeropsis mannii*)
Cucumber seeds	(*Cucumis sativus*)
African Locust seeds	(*Parkia biglobosa*)

Table 2. Fatty acid composition of ingredients from Ghana with potential use in tilapia diets.

	Fatty acids (% by weight)											
	C14:0	C16:0	C18:0	C20:0	C16:1	C18:1	C18:1	C18:1	C20:1	C18:2	C18:3	Total
	(%)	(%)	(%)	(%)	p-oleic (%)	elaidic acid	oleic (%)	vaccenic	(%)	linoleic(%)	alpha(%)	(sum%)
Sesame seed A		10.1		0.6	0.1	6.1	40.6	0.2	0.2	41.8	0.3	100
Sesame seed B		9.9		0.7	0.1	6.3	41.5	0.1	0.2	40.8	0.4	100
Citrullus lanatus **A**		9.8	12.5	0.4			14	0.3		63		100
Citrullus lanatus **C**		10	12.4	0.4			13.3	0.2		63.9		100.2
Cucumeropsis mannii **A**		15.1	7.7	0.4			16	0.3		60.4	0.1	100
Cucumeropsis mannii **C**		16.1	14.4	0.4			15.9	0.3		52.9		100
Cucumis metulferus **A**		15.9	9.8	0.5			31.1	0.4		42.3		100
Cucumis metulferus **C**		14.8	9.3	0.4			25	0.4		50		99.9
Parkia biglobosa **B**		12.9	17.4	4.1			18.2			47.4		100
Parkia biglobosa **C**		12.4	17.8	5.3			17.1			47.3		99.9
Lagenaria siceraria **A**	0.1	13.8	11	0.9			22.1	0.2		51.8	0.1	100
Lagenaria siceraria **C**		13.3	9.7	0.5			20.1	0.2	0.1	55.9	0.1	99.9
Lagenaria siceraria **D**		14	9.8	0.7			21.4	0.3		53.8	0.1	100.1
Cucurbita spp **A**	0.1	15.7	10.2	0.8			35.4	0.3		37.4	0.2	100.1
Cucurbita spp **C**	0.1	13.9	8.4	0.6	0.2		45.3		0.1	31.3	0.1	100

Table 3. Protein content (%) of ingredients from Ghana assessed for potential use in tilapia diets

Sample Description	Protein %
African Locust Seeds A1-1	28.7
African Locust Seeds A1-2	30.4
African Locust Seeds A1-3	30.8
Egushi Seeds A2-1	36.6
Egushi Seeds A2-2	36.1
Egushi Seeds A2-3	36.6
Eggplant Seeds B1-1	37.0
Eggplant Seeds B1-2	37.3
Eggplant Seeds B1-3	37.6
Calabash Seeds B2-1	28.1
Calabash Seeds B2-2	25.5
Calabash Seeds B2-3	29.6
Kidney Beans B3-1	25.5
Kidney Beans B3-2	24.7
Kidney Beans B3-3	24.4
Cucumber Seeds B4-1	39.1
Cucumber Seeds B4-2	38.1
Cucumber Seeds B4-3	38.4
Neri Seeds B5-1	27.1
Neri Seeds B5-2	25.4
Neri Seeds B5-3	25.8
Sesame Seeds B6-1	21.1
Sesame Seeds B6-2	20.8
Sesame Seeds B6-3	20.9

Range: 21 - 39% protein

Table 4. Amino acid composition of ingredients from Ghana assessed for potential in tilapia diets

Units	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**Reference 1**	**Reference 2**
uapb	Citriullus lanatus	Citriullus lanatus	Cucumis metuliferus	Cucumis metuliferus	Cucurbita	Cucurbita	Lagenaria sicerana	Lagenaria sicerana	Lagenaria sicerana	Parika biglobosa	Parika biglobosa	Sesamum indicum	Sesamum indicum	Cucumeropsis mannii	Cucumeropsis mannii	Soybean meal	fish meal (64.5% protein)
Taurine	0.09	0.06	0.12	0.12	0.10	0.08	0.08	0.08	0.09	0.19	0.25	0.04	0.04	0.04	0.03	Not detected	3.55
Hydroxyproline	0.04	0.03	0.06	0.05	0.06	0.04	0.06	0.05	0.05	0.05	0.07	0.03	0.03	0.03	0.03		
Aspartic Acid	1.97	2.11	2.43	2.20	3.20	2.92	2.89	2.61	2.72	2.84	2.68	1.69	1.72	2.42	3.49		
Threonine	0.68	0.73	0.88	0.78	0.86	0.85	1.02	0.91	0.96	0.89	0.86	0.72	0.73	0.87	1.31	2.00	2.64
Serine	0.90	0.97	1.21	1.07	1.49	1.46	1.32	1.15	1.22	1.05	0.98	0.81	0.84	1.08	1.52		
Glutamic Acid	3.55	3.68	4.92	4.52	6.32	5.87	5.33	4.73	5.17	4.63	4.30	3.66	3.81	4.59	6.55		
Proline	0.72	0.79	1.00	0.91	1.11	1.10	1.14	1.00	1.06	1.23	1.14	0.68	0.70	0.94	1.42		
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Glycine	1.33	1.39	1.65	1.52	2.32	2.05	1.89	1.74	1.78	1.24	1.19	1.10	1.11	1.60	1.96		
Alanine	1.00	1.04	1.35	1.25	1.41	1.37	1.43	1.31	1.37	1.24	1.17	0.96	0.99	1.26	1.71		
Cysteine	0.31	0.33	0.41	0.38	0.45	0.41	0.44	0.40	0.41	0.26	0.29	0.38	0.40	0.37	0.49		
Valine	0.99	1.04	1.38	1.32	1.63	1.60	1.50	1.40	1.46	1.38	1.28	1.06	1.08	1.36	1.79	2.70	3.03
Methionine	0.57	0.62	0.51	0.47	0.73	0.70	0.82	0.74	0.79	0.21	0.20	0.55	0.58	0.72	0.87	0.70	1.77
Isoleucine	0.86	0.89	1.21	1.17	1.29	1.29	1.24	1.17	1.23	1.19	1.10	0.82	0.84	1.16	1.55	2.60	2.57
Leucine	1.46	1.53	2.04	1.90	2.23	2.24	2.11	1.92	2.02	1.98	1.84	1.39	1.43	1.88	2.78	3.80	4.54
Tyrosine	0.52	0.61	0.85	0.68	1.19	1.08	0.87	0.74	0.76	0.63	0.62	0.51	0.54	0.71	1.19	1.25	2.04
Phenylalanine	1.17	1.28	1.34	1.19	1.61	1.63	1.74	1.58	1.65	1.29	1.20	0.94	0.97	1.51	2.01	2.70	2.51
Hydroxylysine	0.05	0.05	0.05	0.03	0.04	0.03	0.03	0.03	0.02	0.03	0.03	0.01	0.01	0.03	0.07		
Ornithine	0.01	0.00	0.01	0.01	0.04	0.02	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.03		
Lysine	0.81	0.74	1.15	1.03	1.64	1.46	1.06	1.05	1.07	1.93	1.81	0.63	0.64	0.94	1.21	2.24	4.81
Histidine	0.53	0.56	0.73	0.66	0.81	0.76	0.82	0.75	0.79	0.77	0.73	0.52	0.53	0.72	0.96	1.30	1.78
Arginine	3.00	3.30	4.43	3.91	5.31	5.02	4.93	4.31	4.65	1.78	1.64	2.43	2.54	4.16	5.71	3.60	3.66
Tryptophan	0.38	0.39	0.51	0.48	0.58	0.57	0.54	0.44	0.51	0.11	0.11	0.30	0.31	0.47	0.70	0.70	0.66
Total	20.94	22.14	28.24	25.65	34.42	32.55	31.29	28.13	29.79	24.94	23.50	19.24	19.85	26.88	37.38		
Crude Protein	23.80	24.16	31.06	28.14	39.00	35.28	34.60	30.48	33.22	27.17	27.22	21.16	21.69	30.18	40.01		

Table 5.Composition (% inclusion levels) of experimental diets with different plant lipid sources fed to juvenile *Oreochromis niloticus*

Feed Ingredient	100%Lin (Diet 2)	100%Soy (Diet 3)	LinSoy (Diet 4)	LinPalm (Diet 5)	PalmSoy (Diet 6)
Fishmeal	20	20	20	20	20
Soybean meal	33	33	33	33	33
Wheat bran	34.5	34.5	34.5	34.5	34.5
Soy oil	-	9	4.5	-	4.5
Linseed oil	9	-	4.5	4.5	-
Palm oil	-	-		4.5	4.5
Vitamin & mineral premix	1.5	1.5	1.5	1.5	1.5
Binder	2	2	2	2	2

Table 6. An eight-week growth of juvenile *Oreochromis niloticus* fed experimental diets with different plant lipid sources

Variables	Control	100%Lin	100%Soy	LinSoy	LinPalm	PalmSoy
Av. Iintial Wgt (g)	30.2±2	28.45±1	30.1±1	28.0±4	30.4±1	30.7±4
Av.Final Wgt (g)	58.3±2	47.3±1	53.3±1	50.1±4	50.7±1	52.1±3
Av.Wgt Gain (%)	92.4±2^{a}	66±2^{b}	75.7±2^{b}	79.7±1^{b}	70.3±3^{b}	69.2±1^{b}
Specific.Grt Rate(%/day)	1.32±0.3^{a}	1.02±0.2^{b}	1.1±0.2^{b}	1.2±0.2^{ab}	1.1±0.2^{b}	1.1±0.0^{b}
FCR	1.2±0.1^{a}	1.6±0.2 ^{b}	1.49±0.4^{b}	1.40±03^{b}	1.62±0.2^{b}	1.58±0.6^{b}
Survival (%)	100	98	100	100	98	100

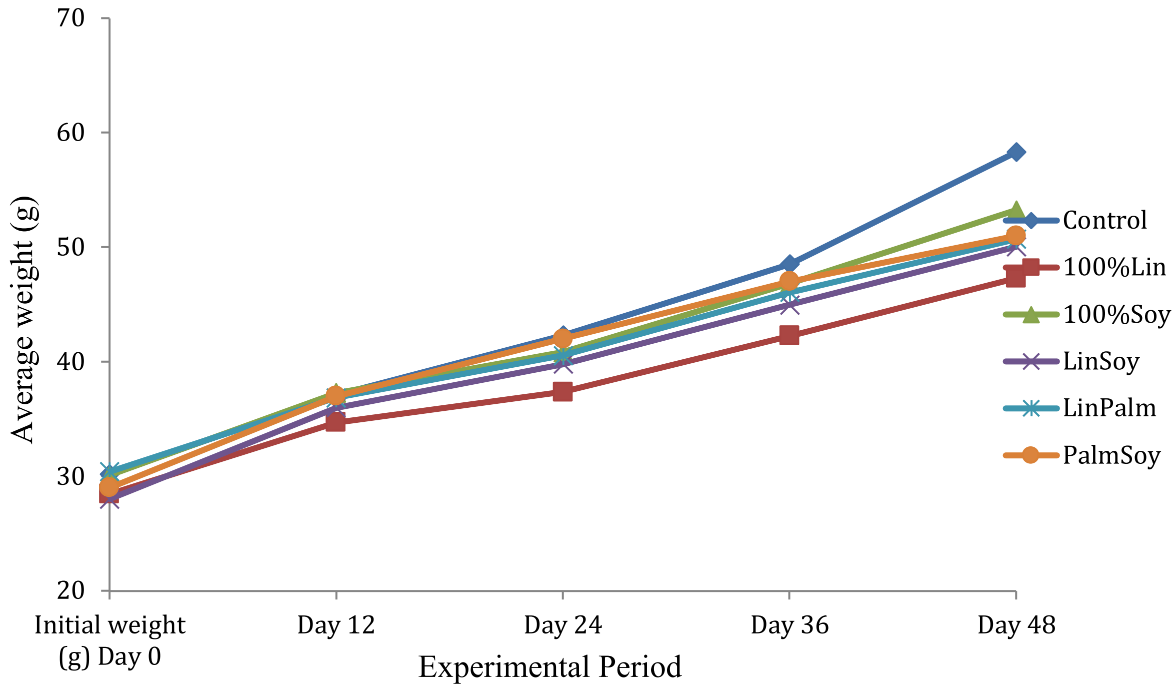

Figure 1. Growth (g) (means ±SD) of juvenile *Oreochromis niloticus* fed experimental diets with different plant lipid sources

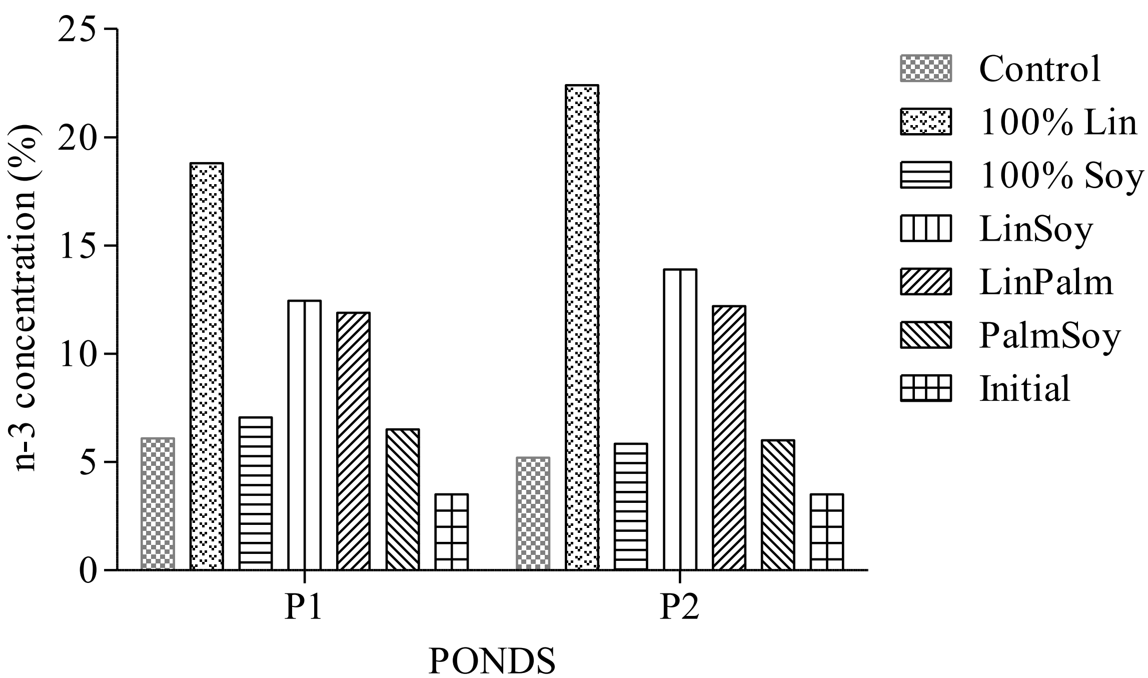

Figure 2. Omega-3 concentration (%) in tissues of juvenile *Oreochromis niloticus* at the end of an 8-week feed trial after being fed with different plant lipid sources

ASSESSMENT OF GROWTH PERFORMANCE OF MONOSEX NILE TILAPIA (*OREOCHROMIS NILOTICUS*) IN CAGES USING LOW-COST, LOCALLY PRODUCED SUPPLEMENTAL FEEDS AND TRAINING FISH FARMERS ON BEST MANAGEMENT PRACTICES IN KENYA

Sustainable Feed Technology and Nutrient Input Systems/SFT/13SFT06AU

Charles C. Ngugi[1], Kevin Fitzsimmons[2], Julius Manyala[3]; James M. Bundi[4], Ann N. Kimotho[5], Judith M. Amadiva[6], Julius N. Ndogoni[6], and Jonathan Munguti7,

[1]*Kenyatta University, Nairobi, Kenya*
[2]*University of Arizona, Tucson Arizona, USA*
[3]*University of Eldoret, Kenya*
[4]*Karatina University, Nyeri, Kenya*
[5]*Ministry of Agriculture, Livestock and Fisheries, County Government of Kirinyaga, Kenya*
[6]*Ministry of Agriculture, Livestock and Fisheries, National State Department of Fisheries, Nairobi, Kenya*
[7]*Kenya Marine and Fisheries Research Institute, Sagana, Kenya*

ABSTRACT

Changing technology in aquaculture has been the major driving force for increasing aquaculture production in the phase of dwindling open water stocks. The choice of technologies and their adoption still remain a focus to increase production, productivity and farm incomes. We assessed the growth of Nile tilapia using locally available materials that included cages and low cost feeds to increased tilapia production in ponds. Various interventions were used among them experimental trials where we formulated low cost diets replacing expensive fish meal. A series of four workshops were also conducted over the course of this project period under this intervention. The first workshop on the development and use of best management practices in aquaculture was held in August 2014. This workshop targeting women in aquaculture had 18 women in attendance. There were two subsequent workshops, held in March and August 2015, and attended by fish farmers from Kirinyaga County. The fourth workshop was held for two days between 2nd and 3rd October 2015. This workshop specifically targeted the youth in aquaculture aimed at training the youth on integrated cage and pond culture focusing on development of cage culture in reservoirs located in the county of Kirinyaga that are presently underutilized by the communities.

Graduate support covered three female students who selected because of their previous linkage with AquaFish CRSP and excellent role they continue to play in development of aquaculture in this region. Among them two have submitted their theses for examination. We submitted five peer reviewed publications during this project period with two being specific on low cost feed and fish meal replacement. AquaFish provided funds for HCPIs and graduate students to attend National, Regional and WAS Meetings and Conferences as well. We note that farmers adopted feed technology very well and in their survey feed formulation and fish breeding were their priority. Under lesson learned, we noted that farmers required frequent visits, simple materials for reading and constant communication. They also require strategies on fish marketing such as use of cell phones applications and Aquashops.

INTRODUCTION

Feeding costs represents about 70% of the operation costs of fish farming. Yet, one of the foreseen constraints to intensification of fish farming is the scarcity of inexpensive and nutritive fish feeds a major driving force for increasing aquaculture production in the phase of dwindling open water stocks (FAO 2006; Gatlin et al. 2007).

Fish meal has traditionally been used as the major protein source for formulated fish feeds because of its high protein content, balanced amino acid profile, good digestibility, high digestibility, palatability, and as a source of essential n-3 polyenoic fatty acids (Lovell 1988; Hardy and Tacon, 2002; Jackson, 2006). But the shortage in world production of fish meal (FM), which is the main conventional protein source coupled with its increased demand in feeds for livestock and poultry is likely to reduce the dependence on it as a single protein source in aquafeeds (El-Sayed, 1998; El-Saidy et al., 2003; Bendiksen et al., 2011). Therefore, the replacement of fishmeal with locally available and cheaper plant feedstuffs is proved to be very essential for the future development of aquaculture sector (Tacon and Forster, 2010). Several fish meal alternatives have been evaluated as possible fish meal substitutes. The results show great variation in the degree of success for partial or complete substitution depending on the species of fish under culture (Kaushik et al. 1995; Kissil et al. 2000; Refstie et al. 2000; Chou et al. 2004; Liti et al. 2006).

Rural pond culture in Kenya is moving from subsistence to small-scale commercial culture; a third intermediate type of aquaculture intervention that is a transition from the rural no cash enterprise to a more commercial aquaculture. Here small-scale commercial farmers endeavor to manipulate stocking densities, and nutrients levels to enhance their economic returns (Quagrainie 2009). Their major production system is the small fishpond that average 400m^2 stocked with Nile tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus*). Since the open pond water can 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. Resulting from success of this practice in south East Asia, Similar models based on the efficiency of cage-cum-pond systems will be introduced with a view to transferring this technology to small-scale farmers in Africa.

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 some farmed fish species, early maturation and breeding before attaining market size is a significant constraint on production. Energy is used for egg production at the expense of growth and, in some cases, such as with tilapia; ponds can become filled with undersized fish. This is a notable problem in Africa with the Nile tilapia. In such cases, stocking with monosex tilapia or sex reversed fish would be useful.

To limit competition for food, raise pond carrying capacity, and increase pond fish production, a 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 and improve their livelihood. This training, therefore, provided the selected farmers with improved skills in seed production and feed formulation skills that would raise their level of fish production within the same area that they have used in the past.

Increased understanding of the basic premises of production of quality seed and feed aquaculture technologies will aid in raising production levels and better managed aquaculture systems. Providing series of workshops to fish farmers was seen as an important contribution towards AquaFish Innovation Labs' overall mission of supporting sustainable aquaculture in this region of sub-Saharan Africa. The aim of these series of workshops was therefore to sensitize fish farmers on factors that increase fish production in ponds by demonstrating use of sex reversal techniques, low cost feeds, introducing methods of suspending cages in ponds and providing farmers an opportunity to commercialize aquaculture in Kenya.

OBJECTIVES

1. Develop low-cost, improved quality feeds utilizing rice bran and freshwater shrimps (*Caridina niloticus*) as fish meal replacement. One Publication.
2. Assess the costs and benefits of three different feeding regimes in cages. Graduate student.
3. Transfer technologies on management of monosex tilapia in cages through training farmers and extension officers. Workshop
4. Training on Best Management Practices for Fish farmers and extension officers.
5. Compare work conducted in this investigation on the use of low-cost supplemental feeds with the accomplishments of 20 years of CRSP-related work in the area.

METHODS AND MATERIALS

In this investigation, we planned on an experiment and a study. The project supported three graduate students instead of two and held three farmers workshops. Additional activity included a fish farmers field day leveraged by FAO regional office and the county government of Kirinyaga. We completed a fish farmer's survey and published two peer-reviewed papers in addition to three that we had submitted earlier. We carried out two experiments on feed trails

Experiment 1. Growth, yields and economic benefit of Nile tilapia (*Oreochromis niloticus*) fed diets formulated from local ingredients in cages: shrimp and bran.

In our endeavour to develop low cost feed for small-scale farmers, we did feed trials in cages. Development of low cost fish feeds through replacement of fish meal as protein source in the diet of Nile tilapia (*Oreochromis niloticus*). This study evaluated the suitability of replacing fishmeal with formulated freshwater shrimp and rice bran diet (Formulated Diet) as the main protein source on growth performance, nutrient utilization, carcass proximate composition and economic benefits in the diet of Nile tilapia (*Oreochromis niloticus*).

Fishmeal was replaced with low value *Caridina niloticus* (Freshwater shrimp) mixed with rice bran at a ratio of 25%, 50%, 75% and 100% and the dietary treatments tested in triplicate for six months. The fish were fed three times a day at 4% body weight. Growth and survival of fish were then recorded and analyses don on the economic benefits of producing Nile tilapia in small cages.

Experiment 2. Growth, yields and economic benefit of Nile tilapia (*Oreochromis niloticus*) fed diets formulated from local ingredients in cages: amaranth.

In our second trails, we used Amaranth leaf as fish meal replacement. The amaranth leaf has been drawing increasing attention for its nutritional content. In addition to growing quickly and being inexpensive to produce, it is also resistant to conditions such as heat and drought. The leaves are documented to contain 17.5-30.3% protein dry matter of which 5% is lysine, while significant levels of Vitamins A and C are also present.

Other major elements in the leaves include sodium (to maintain extracellular fluid balance), potassium (for haemoglobin functioning and maintaining electrolyte balance and normal cellular function), magnesium (for enzyme action, muscular contraction, nerve transmission and bone health) and phosphorus (to regulate acid-base balance and form bone and cells). Amaranth trials are being conducted in various continents and does form part of cutting edge research in fish meal replacement.

Tilapia larvae (mean weight 24.0 ± 2.0 g, 24 day old, mixed sexes) were obtained from Mwea Fish Farm Ltd hatchery. Each of the 15 static earthen ponds with an average surface area of 200 m^2 and an average depth of 1.2 m were stocked with 600 fingerlings. The feeding trials were conducted concurrently in the same set of pond.

Water quality parameters; dissolved oxygen, temperature and pH were determined in all the ponds on daily basis at 0800 and 1800 h. Dissolved oxygen (DO), temperature and pH were measured in situ at each sampling sites, using a calibrated JENWAY 3405 electrochemical analyser (Barloword Scientific Ltd, Essex, UK), with independent probes for each variable. Portions of the water samples were used to determine: (i) nitrite nitrogen (NO2-N) by the sulphanilamide diazotizing method; (iii) Total ammonia nitrogen (TAN) by the indophenol blue method; (iv) soluble reactive phosphorus (SRP) by the standard ascorbic acid method, after filtration of the sample through a 45-μm pore membrane; and (v) total alkalinity (TA) by the acidimetric method, using sulphuric acid as the titrant. Total hardness was also done using EDTA titration. Total phosphorus and total nitrogen were determined following standard digestion procedures.

The strain of *A. cruentus* used for this study was obtained from Mwea Market, Kenya. Four isonitrogenous (38.1% CP) and isocaloric (23.6 kJ kg-1) diets were formulated to contain four inclusion levels (25%, 50%, 60% and 80%) of *A. cruentus* using locally available feeds ingredients. The diets were compared to control feed containing fish meal alone. One-half litre of warm water in which the binder was dissolved was added to each diet thus formulated and mixed. The prepared feed were preserved in a refrigerator (-4°C) until used for feeding fish.

Fish were fed with the standard diet for the first 30 days in the hatchery. They were then transferred to the 15 ponds and stocked at a density of 3 fish m-2. From the day of stocking, which was taken as the 1st day of the feeding experiment, the fish were provided with five experimental diets (D0, D25, D50, D75 and D100) in triplicates per treatment. The fish were hand fed four times a day for the entire experimental period at the recommended body weight of 2.5%. Daily feed ration was determined and adjusted every week based on fish body weights.

Ingredients, experimental diets and fish samples were analysed at the beginning and end of the experiment for crude protein (N2×6.25), crude lipid content, moisture, and ash content using standard methods detailed in AOAC (1995). Gross energy was calculated using conversion factors for protein, lipids and carbohydrates provided in Tacon (1990). Amino acid compositions of the feed ingredients were determined by ion exchange chromatography. Sulphur-containing amino acid were oxidised using performic acid before acid hydrolysis. All analyses were performed, in duplicate, on the sub samples from each pond.

Growth in weight of the fish was expressed as the specific growth rate (SGR, % day-1) using the formula:
SGR (% day-1) = 100 (lnW2 – lnW1)/Δt where: W1 and W2 = initial and final body weights (g) and Δt = time intervals in days.

Survival were determined at the end of the experiment by completely draining the pond and counting the remaining fish in the pond (taking into consideration any fish that died during weighing exercise) and percent survival calculated based on the number of fish remaining in the ponds as a percentage of the stocked fish.

% Survival = number of fish in the tank/ Initial stocking rate

Nutrient utilization was determined using two parameters: protein efficiency ratio (PER) and protein productive value (PPV, %).
PER = (FB – IB) Wprotf-1: PPV = 100 (Wprot2 – Wprot1)Wprotf-1

Where: FB and IB = final and initial fish biomass (g);
Wprot1 and Wprot2 are initial and final protein weight in larvae respectively (g); Wprotf = weight of dietary protein supply per larvae.

RESULTS

Under our experiment trials based on Development of low cost fish feeds through replacement of fish meal with maize gluten meal as protein source in the diet of Nile tilapia (*Oreochromis niloticus*), the best growth performance and feed conversion ratio (FCR) occurred in fish fed fishmeal followed by those fed a combination of rice bran and *C. nilotica*, while rice bran alone resulted in lowest fish growth performance. The best economic benefit was obtained from fish fed a combination of rice bran and *C. nilotica*. We therefore demonstrate that it is possible to replace expensive fishmeal in the diet of *O. niloticus* using combination of cheaper rice bran and *C. nilotica* without compromising economic benefits for the small-scale aquaculturists.

In our study on use of Amaranth leaf as fish meal replacement, we found that although the fishmeal-based diets had a higher profile of various essential amino acids, phenylalanine and tryptophan levels were lower compared to the amaranth leaf. Growth performance was generally unaffected by amaranth leaf replacements, with no significant differences in terms of SGR, mean weight gain and weight gain between diets of 100% fishmeal and those containing 75%, 50%, 40% and 20% of fishmeal respectively. However, the diet containing 100% amaranth leaf protein concentrates produced a lower final weight, weight gain and FCR, and the highest survival rate was observed in fingerlings given 100% fishmeal. Daily feed intake rose with increasing fishmeal substitution, and there were also significant differences in nutrient utilization parameters.

The study concluded that up to 80% of fishmeal could be replaced with amaranth leaf protein concentrates without compromising growth performance and nutrient utilization, but differences between the two were observed. For example, the essential amino acid composition of both was similar except for histidine, leucine, lysine and methionine levels, which were lower in the amaranth leaf. This was presumed to be more limiting for fish growth performance, especially low lysine levels. Anti-nutritional factors such as phytates and oxalates were also observed in the amaranth leaf, with some remaining bound to certain proteins in the diets, rendering them inaccessible to digestive enzymes and thus reducing protein digestibility. This may also have impaired the absorption of some essential amino acids in the amaranth leaf diets, depressing fish growth as a result. Phytate may also have been responsible for reduced growth in fingerlings that were given higher levels of amaranth leaf.

We have gone further to test nutritional composition of locally available ingredients including insects as formulation of possible low cost feeds for small scale farmers. This is being done under a collaborative project with Sterling and Machakos Universities.

Outreach activities- ***Activity 1:***
Based upon the current county and national interest and continuing CRSP efforts on development of aquaculture in Kenya, we organize the BMP women in Aquaculture workshop in August 2014. This workshop, attended by 18 women drawn from Kirinyaga County, was very stimulating and an eye opener for the women involved in aquaculture in this region.

Preparation of the workshop included identifying BMP materials and data generated during the previous BMP project funded under AquaFish CRSP. Government officers, extension workers, and University resource persons were sourced to conduct this workshop. We discussed Best Management Practices in Aquaculture, Gender participation in Aquaculture; the role of women in development of

Aquaculture, record keeping, seed and feed production, and value addition in Aquaculture. Farmers participated in feed formulation, catfish propagation, and gutting and grading fish for local markets.

Activity 2:
Two trainings under this activity were held in March and August 2015. These workshop laid emphasis on techniques on production of monosex Nile tilapia in ponds and formulation of on-farm low cost feed by farmers. The workshops consisted of lecture sessions, demonstration, and on hand spawning/ hatching work. Attendees of the March 2015 workshop totaled 81 and were selected from those farmers that had either been trained by previous CRSP efforts or had benefited from the Government Funded Economic Stimulus Program. The August 2015 workshop had 37 participants drawn from a pool of farmers that had been trained earlier on best management practices.

During the August 2015 workshop, we invited guests from Aller-Aqua Denmark to give a talk on floating and extruded feed and their advantages in fish production as well as advice farmers on how to formulate feeds at a lower cost. Professor Honno from Aller-Aqua gave a stimulating presentation on production of quality fish feed in Denmark. Simple on-farm technologies for propagation of both Monosex tilapia and catfish were demonstrated under the guidance of experienced resource persons from both Kenyatta and Karatina Universities. Farmers were able to evaluate and compare alternative technologies in fish breeding, stocking cages and formulating their own feed at the farm site.

Activity 3:
A youth workshop was held for two days running from 2nd to 3rd October 2015. Attendees were youth from Kirinyaga country drawn from five clusters, extension officer that would later work with the youth, and nine undergraduate students from Karatina University. There five clusters of youth selected based on reservoirs that were identified in the county. Each cluster was responsible for developing and managing cages in their respective reservoir. The County Director of Fisheries selected the youth with assistance of the respective sub county extension officers. They were chosen according to their willingness and ability to participate, and because of suitability of their reservoir as a resource to the community where they were drawn from. A rapid needs assessment was done on the capabilities of cluster farmers, basic requirements such as minimum number of participants, ability to stay as a cohesive cluster, maintain the reservoir, and keep records. For the two days they learned group dynamics and cluster mobilization, cage site selection, production of Nile tilapia in cages suspended in reservoirs or ponds, monosex tilapia production, cage carrying capacity, designing, materials and construction of cages, fish nutrition and feeding fish in cages, sequencing production, and management of cages in reservoirs. At the end of this training, each cluster successfully constructed one cage that they took away with as their first cage.

Activity 4:
An important element of this investigation was a survey to evaluate the alternate technologies we presented to farmers during the workshops. In particular, we sought through a questionnaire detailed feedback from fish farmers who attended the March and August 2015 workshops on their view with regard to the technologies introduced to them and how best they would adapt them. Feedback was also obtained through personal interviews and panel discussions both during and after the workshops. Feedback questions were compiled in electronic form (MS Excel) and analyzed (Tables 1 and 2). We also discussed possibilities of farmers raising fish to 100g then selling to selected farmers who would in turn fatten the fish until market size then send to the local markets. Farmers were enthusiastic with this arrangement and over 15 fish farmers were ready to join in this program.

Additional activities (leverage)
We held a fish farmers' field day organized by the county director of Fisheries, Kirinyaga and attended by the county governor and representative of five fish farmers clusters. There were over 200

attendees present during this event whose climax was learning how to eat fish. Under this investigation we held a side activity that trained 28 young persons for three days on pond construction. We plan on forming a pond construction crew in the county and this team was trained with a view to forming such a crew to be contracted by fish farmers to construct ponds for them whenever such request arises.

Food and Agriculture Organization (FAO) Kenya regional office organized a six days training at Mwea Aquafish Farm attended by 30 Fish farmers. This training is on enhancing commercial hatchery and nursery development under an FAO Kenya/Uganda ASFT (GCP/SFE/001/MUL) project. It is funded for five years and will cover three counties in Central Kenya. Hatchery managers were therefore selected from Kirinyaga, Embu, and Tharaka Nithi Counties. Attendees learned how to select and maintain brood stock, catfish and monosex propagation, biosafety and biosecurity in the hatchery, record keeping, and nursing fry and fingerlings, among other Hatchery management protocols.

Technology transfer and adoption levels

From the survey that we carried out through the questionnaire administered to the attendees, we found out that in most training sessions, the representation of women was 47.8%, compared to that of men who were 52.2% (Table 1). The increased number of female representation is an indication that young women are now equally participating in fish farming. Farmers reported having learnt new fish farming practices from the activity. Ranking high on the list of new ideas learned was monosex seed production, feed formulation, pond management, record keeping, as well as on-farm propagation, and production of catfish fingerlings. Most farmers intend to engage in fry production.

Farmers were keen to learn quality seed production, indicating that farmers recognize there is a shortage of quality fingerlings and would prefer to produce for sale. A large proportion of farmers (80%) realized that aquaculture is a viable enterprise and that they can engage themselves and grow fish at a profit. When asked how much they harvested last season, they reported a range of 10-500 kg per annum. Most farmers, however, promised to increase production after adopting technologies that they learned during the workshops.

As shown in Figure 1, the youngest attendee was 20 years old and the oldest 89 years, with over 80% of the farmers aged between 40 and 60 years; only one farmer was above 80 years old. Farmers interviewed had their education levels ranging from primary to tertiary (Table 2). Twenty-one of these farmers read up to primary level (23.30%), while 34 up to high school and 35 (38.9%) beyond high school, and either acquired a diploma or a degree. Therefore, a majority spoke English fluently, although about 10% indicated that they preferred Kiswahili or English.

Majority of farmers owned more than one pond measuring 300 m^2, and have been in fish farming for more than 3 years. Pond sizes ranged from $200m^2$ to over $1000m^2$. Most of the new farmers had one pond whose size was $300m^2$ because these were the fishponds constructed by the government under the Aquaculture Economic Stimulus Program. They raised Nile tilapia in polyculture with catfish, as Monosex, or kept only catfish as shown in Table 3, and presented better in Figure 2. This implied that although they were being introduced to monosex fish culture, a percentage already are practicing it on their farm.

When asked whether they kept records of their farm activities, only 25% farmers reported that they kept records on stocking and harvesting, but majority acknowledged the need to keep records. Over 60% (25) of the farmers had now joined or formed an association and only 10 indicated that they were willing to be members of Kirinyaga County Fish farmers Association. Most farmers were close to a market, except for 12 farmers who had to travel over 20 kms to get to the markets. On average, most farmers spent not more than Kshs 300 as bus fare to get to the market. Seventy-four percent of these farmers stated that their roads were accessible throughout the year and only 5 said that their roads were impassable, especially during the rainy season.

When asked about information and sources, farmers' replies were interesting, as well as diverse. Most farmers have received information free, and only 4 have paid for information in the past. However, 95% of these farmers were willing to pay for information. They preferred booklets and manuals to posters and fact sheets. Indeed, fact sheets were ranked lowest in order of their preference. Mostly ranked government extension officers as main source of information and universities as the least source of contact. Only one farmer did not have a radio or TV, while majority had both radio and TV. Seventy-six percent of the farmers had a radio each and did value TV programs as main source of information. Interestingly, local NGOs and newspapers did not appear as major source of information.

Farmers prioritized fish and feeding fish, pond management, and pond construction as areas in which they needed information. Other areas included seed production, fish marketing, and equipment, as well as diseases and predator control. They are now using their phones to text messages, while about 18% have access to internet. Their main challenges as stated are lack of information, lack of follow up after training, lack of feed and seed, as well as fish marketing information.

As a way of improving on past fish farming practices, they wished there were more training, workshops, seminars, information dissemination, field visits by extension workers, and aquaculture materials. Certainly, for these farmers and others to adopt aquaculture technologies and have good practices, information sources are needed. Information should be in the form they indicated but not just information, but correct information on aquaculture practices that would contribute greatly to improved pond fish production. They highly valued practical training and information sharing as a way of motivating them and resolving most of challenges they experience in the field.

Feed Technology transfer included Training activities on Best Management Practices Liming fishpond to ameliorate soil acidity, formulating feeds in the back yard, building own cages using locally available materials. During these trainings we laid emphasis on training women and youth in aquaculture practices.

Compare work conducted in this investigation on the use of low-cost supplemental feeds with the accomplishments of 20 years of CRSP-related work in the area

At the onset of the CRSP program, it was evident that fish farmers in Kenya fed their tilapia using expensive poultry diets and dairy meal or bran that were low in protein and contained high fiber content and that there were no commercial fish feed manufacturer in the country. The disadvantages being that commercial poultry rations are not nutritionally balanced for fish, containing more digestible energy per unit of protein than recommended for fish, and brans alone are nutritionally deficient and often unconsumed by the fish due to small particle size. These feeds were fed as powder leading to high losses in the ponds. Pelletizing feeds reduces feed losses, especially when multiple ingredients are included in the formulation.

Work on sustainable feed technology research in Kenya supported by Pond Dynamic Aquaculture CRSP began in 2002 at the Sagana Fish Farm then under the Ministry of Agriculture, livestock and Fisheries, State Department of Fisheries. We planned on generating low cost feed technologies that would help inform future aquaculture feeds research and technology developers beyond the life of the AquaFish program. The purpose of developing low cost feed and teaching farmers to formulate their own feeds using locally available ingredient was aimed and lowering cost of fish production and raising farmers income.

Under work plan nine and ten, experiments done at Sagana prioritized feed formulation using locally available agricultural by-productions. Trials were done using pig finisher, rice, maize of wheat brans in combination with fishmeal to lower the feed cost and provide complete diet. Lower quality pelleted feeds formulated specifically for tilapia, were combined with fertilization regimes to increase the availability of natural food organisms, and found to be economically appropriate approach for intensification of tilapia culture. In an attempt to seek ways of increasing fish yields, a study was conducted at Sagana fish farm to compare locally available rice bran with two compounded feeds, a domestic animal feed (pig finisher pellet) and a laboratory formulated diet. The objective of the this study was to assess economic benefits of a tilapia and catfish polyculture under three different nutrient regimes, namely Rice Bran (RB), Pig Finisher Pellet (PFP) and a Formulated Diet Pellet (FDP), with the goal of establishing recommendations for the most suitable diet for tilapia and catfish fish farmers. The results demonstrated that the two compounded diets had similar nutritional value and promoted better fish growth than rice bran. The fish in this study performed better with formulated diets as compared to single ingredients. Also an economic comparison performed in this study favored the utilization of formulated diets.

The second study was by Dr. David Liti, Karen Veverica and Sagana team on growth and economic performance of Nile tilapia fed on two formulated diets and two locally available feeds in fertilized ponds. Two isonitrogenous (24%) diets were formulated from shrimp meal (*Caridinia nilotica*), cottonseed meal (CSM) and Wheat Bran. One of the formulated diets was supplemented with a locally manufactured commercial vitamin and mineral premix at 0.5% inclusion level. Wheat bran in this diet was included at 63.5% to allow for the premix. Commercial feeds were purchased from a local feed manufacturer while WB was purchased from a wheat-processing factory. Feeding *O. niloticus* on the formulated diets in fertilized ponds resulted in significantly higher mean weight than that of fish fed on commercial diet or Wheat Bran. However, fish that fed on diets formulated with or without vitamins and minerals premix had similar mean weights. Since then, additional trials have been done to replace fishmeal with locally available ingredients and using rice bran as the primary source so as to lower the cost of feed. Results from these trials have been published in various articles listed in the reference section of this report.

QUANTIFIABLE BENEFITS

- Feeding ration estimated to improve farmers' feed efficiency and reduced costs for grow-out of tilapia by as much as 35%, without compromising yield.
- An additional 200 farmers, extension agents, and stakeholders received training through workshops and farm visits.
- Peer-reviewed publications and presentations and regional and international conferences increase AquaFish visibility and shared technologies
- Three graduate students received research training and education on sustainable aquaculture technologies and have since then submitted their theses for examination except one who suspended here studies due to family issues.
- Linkages increased numbers of farmers trained and technology transfer
- Annual meetings increased level of interaction and possibilities of furthering collaboration among host country PIs various international organizations

LESSONS LEARNED

During this investigation, and as has been the case in the past, the project team enjoyed a successful four-way partnership between the Government Ministry responsible for Fisheries and Aquaculture the County government in the Host country, the University hosting this project, and The US Host University. This partnership has worked well to get work done and seems to have had the beneficial effect of bringing the partners into a good working relationship, wherein they work together towards common goals in aquaculture development and in training of extension personnel and farmers. We need to recall that in the recent past (2010) Kenya adopted a new constitution that devolved all agricultural function to County governments. Consequently, most of collaboration is now at the County level and this has brought new challenges in the way we communicate and engage government extension officers.

In Kirinyaga County, we began engaging the County Governor, and later the County Chief officers, into our programme. This has made our work much easier, and whenever we wish to invite farmers we do so through the County government and have not experienced any major challenge. Indeed, we began by inviting the governor and his executives to our meeting so as to solicit support and leverage funding. The governor spent a whole day with fish farmers, ate fish with us, and donated five freezers to cluster farmers. This was very encouraging and did give us an easy take off as well as open channels of communication with County government.

CONCLUSION

We have been training farmers on various aspects of fish farming. However, in the recent workshop we challenged farmers to give thought to the level of investment we have put in these trainings and that in future we shall require some accountability; mainly though reporting on how much production they have achieved and proof that our investment in training is changing their incomes to what extent. One way will be by evaluating their management performance and increased fish yield. We will then be able to assess ourselves and determine what we have achieved and to what extent. We therefore laid more emphasis on record keeping and looking at aquaculture as a business.

Through linkages, we received leverage funding from FAO to run training for six days for Hatchery managers and one day workshop organized by Ana Athifah and Nathaniel Hishamunda. FAO has a five-year project in this region and is indeed riding on the CRSP success as well as the government Economic Stimulus programme. Both have had links with AquaFish Innovation Lab and so were willing to partner with us. Though the 5 publications and numerous fact sheets generated from this project since 2013 we are reaching out more investors and fish farmers in the region. Farmers in this region (Kenya, Uganda and Tanzania) have now in place National Aquaculture Associations that have regular annual meetings. These associations are ready to engage government and policy makers in discussions that will provide legislation and budget for aquaculture legal framework and financial support.

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TABLES AND FIGURES

Table 1. Summary of responses to the question on gender

Response	Number	Percent
Female	43	47.8
Male	47	52.2
Total	90	100.0

Table 2. Summary of responses to the question: "what is your level of education?"

Response	Number	Percent
Primary	21	23.3
Secondary	34	37.8
Tertiary	35	38.9
Total	90	100.0

Table 3. Summary of responses to the question "What is your method of fish culture?"

Response	Number	Percent
Tilapia Monosex	28	31.1
Tilapia with Catfish	22	24.4
Catfish	5	5.6
Tilapia Mixed sex	30	38.9
Total	90	100.0

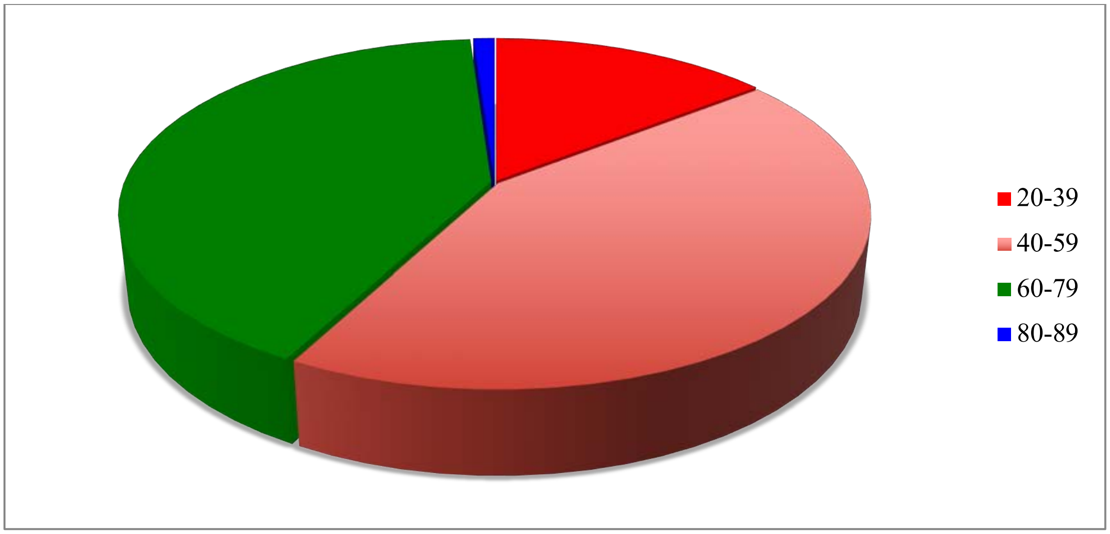

Figure 1. Summary of age groups of fish farmers that attended the workshops

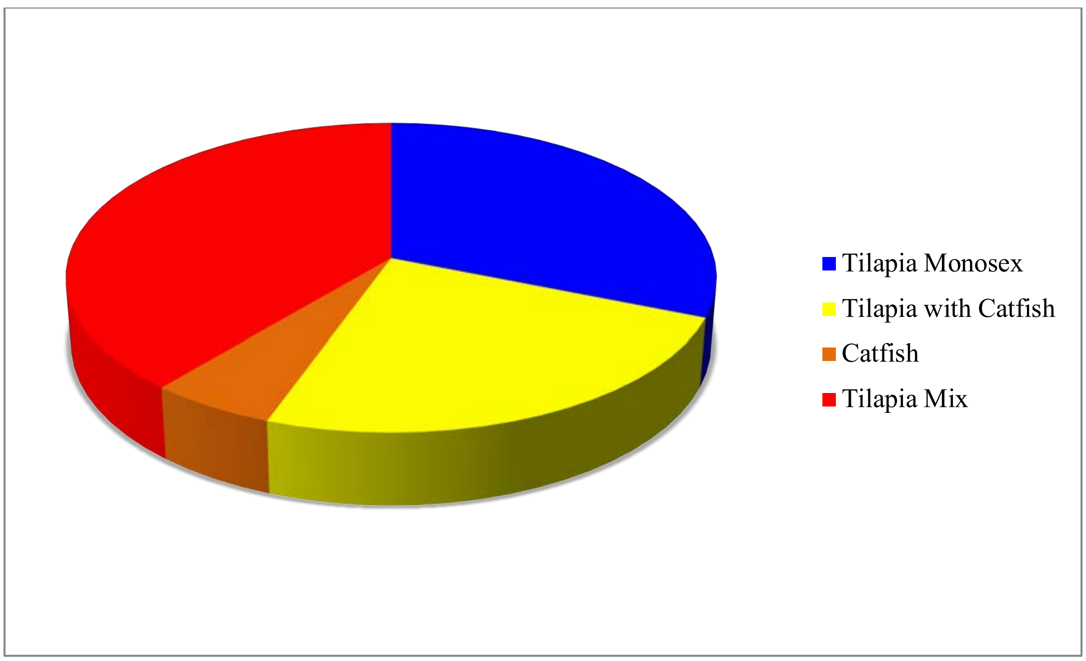

Figure 2. Summary of fish farmer aquaculture practices

Fish farmers Hatcheries in Kenya are using tilapia egg incubation technology to raise fry in the hatchery using locally available materials.

Joe Molnar and Ram Bhujel listen to a presentation during the AquaFish session in Cape Town South Africa.

The AquaFish team Visited fish farmers that were trained on feed formulation to check on how they were formulating and feeding their tilapia.

The AquaFish team in a technical session in Kampala regional meeting, Uganda;

Ann Athifah and Nathaniel Hishamunda both from FAO and the AquaFish team conducted a Business Training for fish farmers at Mwea Aquafish farm.

TOPIC AREA

CLIMATE CHANGE ADAPTATION: INDIGENOUS SPECIES DEVELOPMENT

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SUSTAINABLE SNAKEHEAD AQUACULTURE IN CAMBODIA

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND01UC

So Nam[1], Nen Phanna[1], Chheng Phen[1], Bui Minh Tam[2], Pham Thanh Liem[2], Nguyen Hong Quyet Thang[2], Tran Thi Thanh Hien[2], Pham Minh Duc[2] and Robert Pomeroy[3]

[1]Inland Fisheries Research and Development Institute, Cambodia
[2]College of Aquaculture and Fisheries, Can Tho University, Vietnam
[3]Department of Agricultural and Resource Economics/CT Sea Grant, University of Connecticut-Avery Point, Groton, CT, USA

ABSTRACT

The Cambodian government banned snakehead aquaculture in 2004 due to unsustainable methods. Recent research in Vietnam led to sustainable practices there. As part of technology transfer to Cambodia, a study was conducted to compare survival and growth of domesticated snakehead from Vietnam (VN) with those of non-domesticated snakehead from Cambodia. Cambodian broodstock fish were collected from Mekong River (MR) and Tonle Sap (TS), and F1 Cambodian fish from previous breeding (F1) were also used. Larvae from spawns of four broodstocks (VN, MR, TS, and F1) were subjected to a weaning protocol developed in Vietnam in a 60-d hatchery phase, followed by a 6-month grow-out in ponds. VN fish grew significantly faster than Cambodian fish in the hatchery, followed by continued rapid growth in the grow-out phase, largely due to increased feed consumption. Cannibalism rates ranged from 40-42% in the hatchery phase except for MR fish (57%) and 12-45% (VN lowest, MR highest) in the grow-out phase. It is not known whether differences are due to inherent genetic differences between wild Vietnamese and Cambodian fish, or to selective breeding (intentional or not) in Vietnam. Results will inform Cambodian aquaculture policy following the lifting of the ban in 2016.

Also, an experiment was conducted in Vietnam to assess spawning of various broodstock types (domesticated male x domesticated female; wild male x wild female; wild male x domesticated female and domesticated male x wild female) and the subsequent survival and growth of their offspring. Domesticated broodstock were generally larger than wild broodstock. Fecundity, fertilization rate and hatching rate of wild x wild fish were higher than those for any of the other treatments. Egg diameter and larval length at hatch and at yolk absorption were also greater for the offspring of the wild x wild fish cross than for other treatments. Survival of fingerlings after 45 days was slightly higher for offspring of the domesticated x domesticated cross, but growth was greatest in the offspring of the wild male x domesticated female cross.

INTRODUCTION

In Cambodia wild snakehead were traditionally cultured in small cages and ponds prior to the snakehead aquaculture ban (see below). Feed represented more than 70% of the total operational cost and the main type of feed for wild snakehead culture was small-sized fish (SSF), representing 60 to 100% of the total feed used depending on feeding strategies adopted by different farmers. During the dry season (October to May), the most important source of feed was freshwater SSF, while more marine SSF species were used during the rainy season (June to September). Importantly, the snakehead production contributed more than 70% of total aquaculture production in Cambodia due to its popularity as food. Snakehead still has high market and trade demand in Cambodia as well as in Viet Nam, being found in most Cambodian and Vietnamese dishes at all wealth class levels (i.e. poor, medium and rich people). During the first phase of AquaFish CRSP (2007-2009), the study revealed that 33 species of freshwater SSF in the Viet Nam Mekong Delta (Hien et al. 2015) and nearly 200 species of SSF in the Cambodia Mekong basin (So et al. 2009) were detected in the supply of SSF for snakehead culture in Vietnam and Cambodia, respectively, including juveniles of commercially important fish species. Overall, SSF contribute more than 70% to total freshwater capture fisheries production in Cambodia (So et al. 2009).

The government of Cambodia put a ban on snakehead farming in September 2004 by the Announcement No. 4004. 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, freshwater SSF have illegally been exported to Vietnam for feeding the significantly and commercially developed snakehead aquaculture in Vietnam. The first phase study of AquaFish CRSP (2007-2009) indicated that the incentives for choosing snakehead over 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 not only resulted in positive impacts on poor consumer group's from increased availability of freshwater SSF in Cambodia, but also provided negative effects on food and nutrition security and livelihood of tens of thousands of snakehead farmers who depended on this livelihood for improving household food and nutrition security and 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 increased in recent years in order to supply local and external markets. In order to remove this ban, the same announcement mentioned that successful technologies of domesticated breeding, weaning and rearing or growing-out of snakeheads using formulated diets should be developed and made applicable at on-station and on-farm levels in Cambodia.

During the second phase of AquaFish CRSP (2009-2011), wild striped snakehead *Channa striata* broodstocks were successfully developed, matured and semi-artificially induced to spawn using the hormone HCG on-station in Cambodia. The *C. striata* aged 30 days old after hatch could gradually and successful accept AquaFish CRSP Snakehead Formulated Feed developed by AquaFish CRSP project as replacement of SSF at the rate of 10% every three days for a period of 30 days of feeding; and during the third phase of AquaFish Innovation Lab (2013-2015), the wild snakehead larvae aged 17 day-old after hatch could gradually wean (using the protocol adopted from Hien and Bengtson 2009; 2011) and accept the formulated feed. These fish were grown from larvae to adult fish (as F1 generation) at FARDeC by feeding them with pelleted feed developed by the AquaFish CRSP project (Nen et al., 2015). F1 mature adult snakehead will be developed into F2, F3and F4 generations to optimize the growth and survival rate while feeding on formulated, pelleted feed to ensure the efficient production and economic benefit, in order to release the ban on snakehead aquaculture and to sustain this aquaculture industry in Cambodia without negatively affecting the wild SSF populations. As snakehead culture in Vietnam becomes more domesticated, there is concern about the possibility of inbreeding in hatchery broodstocks. A method is needed to try to minimize inbreeding. We conducted a breeding experiment at CTU between wild and domesticated snakehead collected in Vietnam. If the off-spring from this back-cross perform as well in hatchery conditions as the same domesticated parent, back-crossing could be a good strategy to reduce potential inbreeding resulting from long-term domestication.

This study contributes to the goal of the overall project, which is enhanced trade and investment for global fishery markets and improved nutrition and food security through sustainable aquaculture development in Cambodia, especially to overturn the ban on snakehead aquaculture in Cambodia. The government required research to develop a sustainable process to raise farmed snakehead without doing harm to the wild fishery on which so many people depend. There is a great potential for enhanced trade in snakehead fresh and processed forms and investment for snakehead in Cambodia after the ban is released. In addition, if the wild snakehead fishery is sustainably managed after releasing the ban, there is also good potential to increase the trade and marketing of fresh and processed forms of wild snakehead with other Mekong riparian countries such as Thailand, Lao PDR and Viet Nam, and other countries in Asia, Europe, America and Australia. As a result, household food security, nutrition and income of tens of thousands of snakehead farmers who depend on snakehead aquaculture in Cambodia will be improved through sustainable aquaculture development and aquatic resources management.

The objective of the study was to focus on optimization of the domestication and development of F1 into F2 snakehead in Cambodia as comparing to wild snakeheads collected from five different natural water bodies in Cambodia and domesticated-hatchery snakehead collected from Vietnam in regard to weaning and grow-out on formulated or pelleted feed. Furthermore, the assessment of economic efficiency during grow-out was also conducted to provide technical and policy recommendations for sustainable snakehead farming as well as lifting the snakehead ban in Cambodia.

OBJECTIVES

To continue the domestication and development of Cambodian snakehead *Channa striata* brooders, specifically:

- To compare growth performance and survival rate of different snakehead strains regarding to weaning and grow-out experiments; and
- To compare economic efficiency of grow-out experiments of the different snakehead strains.

MATERIALS AND METHODS IN VIETNAM:

Wild snakehead from U Minh Wetland (U Minh dist., Cam May province) were collected and conditioned in the hatchery in Cantho University, Vietnam. Domesticated snakehead were purchased from a hatchery in An Giang (Chau Phu dist., An Giang provice). They were trained via feeding with commercial pellet containing 40% crude protein for 2 months before breeding.

Induced spawning using HCG hormone

All snakehead broodstock fish were checked monthly for egg maturation based on the method of Nikolsky (1963). Broodstock with egg maturation at the same stage were selected for induced spawning in order to simultaneously produce batches of larvae for the weaning experiment. Male and female broodstock from wild and domesticated sources were stimulated with HCG and distributed randomly in fiber tanks (0.3 m3/tank). The cross breeding procedure is presented in Table 1. Each treatment was replicated with 10 pairs.

Weaning method

After yolk absorption at 3 days after hatching (dah), larvae from each treatment were fed with live *Moina* for 7 consecutive days till 10 dah, and then larvae were fed with a mixture of dead *Moina* and ground freshwater small-sized fish (replacing *Moina* by 20% per day) for 7 days more till 17 dah. Larvae were weaning onto formulated feed beginning at 17dah with replacement of small-size fish by 10% per day until freshwater small-sized fish werecompletely replaced by formulated feed. Formulated feed contained 40-50% crude protein (Hien et al., 2016 and Hien et al., 2017).

Statistical analysis
Significant differences ($p<0.05$) among the treatments in growth and survival rate and feed intake of snakehead larvae were determined by one- way ANOVA. Tukey's HSD test was used to determine specific differences among means where appropriate.

RESULTS IN VIETNAM:

Spawning results
The weight of wild strain fish (from U Minh wetland) varied from 0.3-0.6 kg/ind and domesticated strain (from farms in Chau Phu, An Giang) were 0.5-0.8 kg/ind. Spawning parameters are presented in Table 2.

Values of fecundity, fertilization rate and hatching rate of treatment 3 (Wild female x wild male) gave the highest results compared to other treatments, although the spawning rate was lower (Table 2). So the egg quality of wild snakehead are still better than with domesticated snakehead. Egg diameter in treatment 3 (Wild female x wild male) was higher than other treatments, along with the length of larvae at hatching and after yolk absorption (Table 3).

Larva rearing
After nursing 45 days, fingerling were collected to weigh and measure individually. The results are shown in Tables 4 and 5. Growth of larvae from crosses between wild males and domesticated females was better than that of other crosses. Survival of treatment 1 (domesticated female x domesticated male) gave the highest value (45.5%) compared to treatments 2, 3 and 4 at values of 41.3, 41.0 and 42.0%, respectively at $p<0.05$.

CONCLUSIONS

- After conditioning, snakehead breeders from wild and domesticated sources spawned with high rate (90-100%). Spawning parameters of wild snakehead source have high value.
- The survival rate of fingerlings from domesticated source is higher ($p<0.05$) than wild snakehead. However, the growth of fingerlings from wild female crossed with domesticated male is higher than that of other crosses.

MATERIALS AND METHODS IN CAMBODIA:

The experiment, consisting of weaning and grow-out phases, was conducted at the Freshwater Aquaculture Research and Development Center (FARDeC), Prey Veng province, Cambodia under the direct supervision of the Inland Fisheries Research and Development Institute (IFReDI). In addition to available breeders already at FARDeC (referred to here as F1, the offspring of Cambodian wild-caught fish), adult wild *C. striata* from different natural water bodies of Cambodia (Tonle Sap and Mekong River) were collected and conditioned for spawning at FARDeC. Domesticated snakehead were also purchased from a hatchery in Can Tho, Vietnam, and also conditioned at FARDeC for induced spawning to produce larvae for the experiment. Snakehead at that Vietnamese hatchery have been reared through severalgenerations, although there has not been a formal selective breeding program (T.T.T. Hien, personal communication).

All snakehead broodstock were checked monthly for egg maturation based on the method of Nikolsky (1963). Broodstock fish with egg maturation at the same stage were selected for induced spawning with HCG hormone in order to simultaneously produce batches of larvae for the weaning experiment. Those batches were used for weaning treatments to compare their growth performance and survival rate on pellet feed using the optimum weaning protocol for *C. striata* (So et al., 2011; Hien et al. 2017) from live *Moina sp.* to formulated feed, as follows.

After yolk absorption at 3 DAH, larvae were fed live *Moina* for 7 d till10 DAH, and then fed a mixture of dead *Moina* and ground SS fish (replacing *Moina* by 20%.day-1) for 7 d more till 17 DAH. The experiment began at 17DAH with replacement of SS fish by 10%.day-1 until SS fish were completely replaced by formulated feed made by Dr. T.T.T. Hien at Can Tho University, Vietnam for consistency with the diet used by Hien et al. (2017) and replicated by IFReDI and FARDeC research teams in Cambodia (So et al., 2011).

The experiment consisted of four treatments with six replicates each, with larvae originating from the four broodstock groups: F1 (broodstock were offspring of wild-caught Cambodian fish), Mekong (broodstock were wild-caught from the Mekong River in Cambodia), Tonle Sap (broodstock were wild-caught from the Tonle Sap in Cambodia), Vietnam (broodstock were purchased from a Vietnamese hatchery after several generations of domestication there). All treatments were subjected to the same weaning protocol, as mentioned above. Larvae were stocked in 100-L tanks with stocking density of 5 fish.L^{-1}. The fish were fed to satiation by hand twice daily. Any uneaten feed and feces were siphoned out before feeding.

Fish mortality, food consumption and water quality (temperature, pH and dissolved oxygen) were recorded daily. Larvae were sampled, weighed and measured at biweekly intervals. At the end of the weaning experiment, final wet body weight (FBW, mg), wet weight gain (WWG, mg) and survival rate were determined. Cannibalism rate was calculated from the number of fish stocked into each tank, minus number of known (i.e., removed) mortalities, minus number of fish remaining at the end of the experiment, divided by the number of fish stocked and multiplied by 100%.

Immediately following the weaning phase, fish from each treatment and replicate of that phase were transferred to corresponding treatments and replicates in ponds at FARDeC for the 6-month grow-out phase of the study. Each replicate was now contained in a 3 m x 1 m x 1.5 m hapa net. Fish were fed commercial pelleted feed (40% crude protein) to satiation by hand twice daily at 09:00h and 16:00 h. The amount of feed consumed by fish and fish mortality were recorded daily. Water qualities (temperature, pH, dissolved oxygen, NH_3 and NO_2) were monitored weekly. Fish growth was measured monthly with 30 sampled fish. The survival rate and cannibalism rate were determined at the end of experiment. At the end of the experimental grow-out phase, we also calculated the economic efficiency (profit) of the snakehead in different strains.

Data were subjected to analysis of variance, followed by Tukey's HSD test to determine treatment differences using SPSS 16.0 and differences were considered significant at $p<0.05$. *Data calculation*
SR (%) = (Numbers of fish at the end of experiment/ numbers of initial fish) x 100
WG (g) = Final body weight – Initial body weight
DWG (g fish-1 day-1) = (Final body weight- Initial body weight)/ number of experimental day
SGR (% of body weight day-1) = ((ln final weight — ln initial weight)/ number of culture day) x100
FI (g fish-1 day-1) = (Feed intake/no. fish)/ number of experimental day
FCR= Feed intake/ Weight gain
Profit (KHR; Khmer Riel) = Total income-total cost

RESULTS IN CAMBODIA

Hatchery phase

Snakehead larvae from the domesticated Vietnamese broodstock grew significantly more during the experiment than did larvae from any of the other three treatments, which did not differ among themselves (Figure 1a, Table 6). The greatest difference in growth between the Vietnamese fish and the Cambodian fish happened between days 40 and 60 of the experiment, although Vietnamese fish were already significantly larger than the Cambodian fish by day 40 (Figure 1a). The better growth of the Vietnamese fish appeared to stem primarily from greater feed intake (Figure 1b), but their FCR was not significantly different from that of the Tonle Sap or F1 fish; only the Mekong fish FCR was significantly higher (Table 6). Survival rate of Tonle Sap and F1 fish was significantly higher than that of Mekong and Vietnamese fish (Table 6). Cannibalism was by far the largest cause of mortality and Mekong fish had a significantly higher cannibalism rate than did the other three treatments, which did not differ among themselves (Table 6).

Mortality from "natural causes" (i.e. fish found dead and removed from tanks during the experiment) was very rare.

Grow-out phase

Vietnamese fish continued their significantly greater growth in the grow-out phase, reaching a weight of about 340 g after 6 months, compared to weights of about 140-150 g for all the Cambodian treatment groups (Figure 2a, Table 6). Survival was highest for Vietnamese and F1 treatments, less so for the Tonle Sap treatment, and lowest for the Mekong Treatment, with cannibalism still contributing more to mortality than non-cannibalism causes (Table 6). FCR was not significantly different among treatments (Table 6), although feed intake of Vietnamese fish was significantly higher (Figure 2b). Yield.hapa^{-1} was significantly greater for the Vietnam fish than for any of the Cambodian fish (Table 6).

Economics

The economics of experimental snakehead is presented in Tables 7 and 8. In this experiment, since feed cost accounted for more than 70% of the total expenses, we focused only on feed cost (4200 KHR/kg) and income from selling snakehead on farm-gate price (8000 KHR/kg) in the current market to make the economic analysis. The Mekong strain showed lost profit (-1400 KHR/kg fish produced). The highest profit was attained in the Vietnamese domesticated strain (2000 KHR/kg fish produced), followed by Cambodian F1 domesticated strain and Tonle Sap strain (1600 KHR/kg fish and 300 KHR/kg fish, respectively).

DISCUSSION

To the best of our knowledge, this is the first demonstration of differences in weaning performance based on differences between larvae from wild-caught vs. domesticated broodstock. Fish from the Vietnamese broodstock appear to grow more (probably due to higher feed intake) while maintaining an acceptable FCR, although their cannibalism rate was the same as larvae of most of the wild-caught strains. This observation has multiple possible explanations. The most obvious is that domestication in Vietnam has produced a line of fish that have been selected over time (not necessarily intentionally by the hatchery managers) for greater feed consumption and growth. Given the high rates of cannibalism seen in all the treatments of our experiment, one can imagine that maximization of feed consumption and growth would be a trait for which selection occurs in a hatchery. Another explanation is that, even prior to domestication; wild fish in Vietnam were genetically different from those in Cambodia and could inherently grow faster, regardless of domestication. The finding that the fish in the F1 treatment in our experiment were similar to fish in the other treatments derived directly from Cambodian wild-caught fish suggests that any selection in the hatchery does not occur in just one generation. Finally, the observation that the cannibalism rate in domesticated fish from Vietnam was the same during weaning as that of fish from wild-caught Cambodian broodstock suggests that, even though selection for high-consuming, fast-growing fish may be a by-product of domestication, it apparently does not reduce the variance in growth rates among individuals that makes cannibalism possible.

Given the results of the weaning phase, it was not surprising that the Vietnamese fish would continue their rapid growth during the grow-out phase. Presumably, the same arguments made above for growth differences between Vietnamese and Cambodian fish apply to grow-out as well. Further examination of genetic differences would help elucidate the basis for growth differences. Selective breeding programs for increased growth have greatly benefitted the finfish aquaculture industry, especially for Atlantic salmon (*Salmo salar* L. 1758), but for many other species as well (Gjedrem et al. 2012). Thus, domestication and breeding of Cambodian sources of *C. striata* may one day result in production similar to that seen for Vietnamese fish in these trials.

Based on research done in Vietnam to change aquaculture of *C. striata* from SS fish-based to pellet feed-based (Hien et al. 2015b, 2016) and technology transfer from Vietnamese to Cambodian scientists, the Cambodian government lifted the ban on snakehead in 2016. More research and policy development will be required before the Cambodian snakehead aquaculture industry achieves performance like that seen in Vietnam. Development of hatcheries, feed mills, and processing and distribution facilities are required for industry development. Results of the current study will help to inform further research and policy development to enable sustainable snakehead aquaculture in Cambodia.

CONCLUSIONS

The study concludes that both Viet Nam hatchery snakehead (domesticated) and Cambodia indigenous wild snakehead (non-domesticated) can accept formulated feed, with similar product quality. However, Vietnam hatchery snakehead show higher growth rate and profit than Cambodia wild capture snakehead and F1 generation because Vietnam hatchery snakehead has undergone domestication and selection breeding for more than 10 years. Considering economic efficiency, replacing freshwater small-sized by formulated feed up to 100% is possible for both Vietnamese domesticated snakehead and Cambodian domesticated snakehead, however, the domesticated snakehead shows higher profit than Cambodian domesticated snakehead.

Recommendations

The following recommendations should be carefully considered for policy and action plan development in order to achieve sustainable development snakehead aquaculture in Cambodia:

- The availability of good quality hatchery broodstock has very important implications for protecting the SSF that are usually fed to snakeheads.
- Sufficient numbers of broodstock should be developed at hatcheries through selective breeding and weaning techniques to produce high-quality seed.
- Survival and growth rate of Cambodia indigenous snakehead should be optimized through development of F2, F3 and F4 generation broodstocks and genetic selection of wild capture snakehead collected from different natural water bodies in Cambodia.
- Practical formulated diets should be developed for snakehead broodstock, fry and fingerlings to replace SSF from capture fisheries
- Extension services should be provided to farmers on techniques for snakehead breeding, weaning and grow-out using formulated diets
- Government, business and development partners should be encouraged to invest in the value chain of snakehead aquaculture development, especially the private sector to formulate and improve commercially manufactured feed that is better integrated into local economy with fewer imported ingredients and lower prices.

QUANTIFIED ANTICIPATED BENEFITS

This research provides information on domesticated breeding, weaning and growing out of snakehead fish, especially development of Cambodia's snakehead aquaculture technologies, in order to lift the ban on snakehead culture in Cambodia. The following are quantifiable anticipated benefits:

- Scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staffs, private sector and university lecturers and students working on the issues of snakehead aquaculture in Cambodia as well as in other Mekong riparian countries

were better informed about research methods and findings, and have better recommended policies and strategies for sustainable snakehead aquaculture.
- Three undergraduate students were supported and trained by this investigation through their B.Sc. thesis research.
- Benefits to the US include improved knowledge and technologies on domestication of freshwater fish species for aquaculture and this aquaculture is considered as a climate change adaptation measure.

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TABLES AND FIGURES

Table 1: Cross breeding between wild and domesticated snakehead in Vietnam

Treatment	Gross breeding
1	Domesticated female (♀) x Domesticated male (♂)
2	Domesticated (♀) x Wild male (♂)
3	Wild female (♀) x Wild male (♂)
4	Wild female (♀) x Domesticated male (♂)

Table 2. Spawning parameters of different snakehead in Vietnam

Treatment	Spawning rate (%)	Fecundity (egg/kg)	Fertilization rate (%)	Hatching rate (%)
1 (DxD)	100	35,121	71.5±27.7	39.7±32.3
2 (DxW)	100	36,012	85.7±24.6	70.2±28.5
3 (WxW)	90	54,567	86.7±32.6	92.4±9.5
4 (WxD)	100	42,115	67.1±39.9	66.4±36.2

Note: D; domesticated; W: wild

Table 3. Abnormal rate, egg diameter and length of larvae of different snakehead in Vietnam

Treatment	Abnormal rate (%)	Egg diameter (mm)	Length of larvae (mm)	
			At hatching	After yolk absorption
1 (DxD)	6.5	2.75	6.08±0.38	11.37
2 (DxW)	8.5	2.78	5.81±0.43	11.40
3 (WxW)	1.2	2.80	6.01±0.40	11.67
4 (WxD)	2.2	2.72	5.87±0.43	11.69

Table 4. The growth of fingerling from different snakehead in Vietnam

Treatment	Initial length (cm)	Final length (cm)	Initial weight (g)	Final weight (g)
1 (DxD)	0.608	4.61±0.78	0.0021	1.15±0.54
2 (DxW)	0.581	4.66±1.17	0.0020	1.13±0.63
3 (WxW)	0.601	4.59±0.87	0.0019	1.04±0.50
4 (WxD)	0.589	5.12±1.18	0.0020	1.50±0.81

Table 5. Survival rate of different snakehead fingerling from different in Vietnam

Treatment	Survival rate (%)
1 (DxD)	45.6±16.9[a]
2 (DxW)	41.3±10.0[a]
3 (WxW)	41.0±15.5[a]
4 (WxD)	42.0±11.8[a]

Data are means of three observations ± SE. Means in the same column with the same superscript are not significantly different (P<0.05).

Table 6. Survival and growth of *Channa striata* of Vietnamese and Cambodian origin during weaning (hatchery phase) and grow-out phases of production. Both survival rate and cannibalism rate represent percentages of all the fish stocked in a given treatment. FCR is feed conversion rate. Values (mean ± SE) in a column followed by the same letter are not significantly different.

	Survival rate (%)	Cannibalism rate (%)	Weight gain ($g.fish^{-1}$)	FCR	Yield.$hapa^{-1}$ (kg)
Hatchery phase					
Vietnam	41.6 ± 3.4 B	41.1 ± 3.1 B	10.88 ± 0.47 A	1.63 ± 0.11 A	-
Tonle Sap	55.8 ± 2.9 A	42.0 ± 3.0 B	4.66 ± 0.50 B	1.91 ± 0.11 A	-
Mekong	39.0 ± 3.8 B	57.2 ± 4.0 A	3.24 ± 0.43 B	3.82 ± 0.86 B	-
F1	53.8 ± 3.6 A	40.2 ± 3.2 B	4.96 ± 0.46 B	2.06 ± 0.09 B	-
Grow-out phase					
Vietnam	80.8 ± 4.4 A	12.3 ± 4.0 A	324.2 ± 8.0 A	1.57 ± 0.06 A	81.0 ± 3.6 A
Tonle Sap	67.0 ± 5.6 A, B	27.4 ± 5.3 B	148.1 ± 16.4 B	1.90 ± 0.08 A	27.3 ± 1.93 B
Mekong	50.7 ± 9.4 B	45.1 ± 8.8 B	132.9 ± 19.0 B	2.08 ± 0.24 A	17.4 ± 2.91 B
F1	77.6 ± 5.0 A	21.0 ± 4.7 A	147.2 ± 17.7 B	1.59 ± 0.05 A	35.0 ± 3.44 B

Table 7. Economics of experimental snakehead cultured per hapa (3m x 1m x 1.5m)

Treatments	Total cost (feed) (thousand KHR/hapa)	Total income (thousand KHR/hapa)	Profit (thousand KHR/hapa)
Vietnam	512	648	136
Tonle Sap	209	218	9
Mekong	160	139	-21
F1	225	280	55

Table 8. Economics of experimental snakehead cultured per kg fish produced

Treatments	Total cost (feed) (thousand KHR/Kg fish)	Total income (thousand KHR/Kg fish)	Profit (thousand KHR/Kg fish)
Vietnam	6	8	2
Tonle Sap	7.7	8	0.3
Mekong	9.4	8	-1.4
F1	6.4	8	1.6

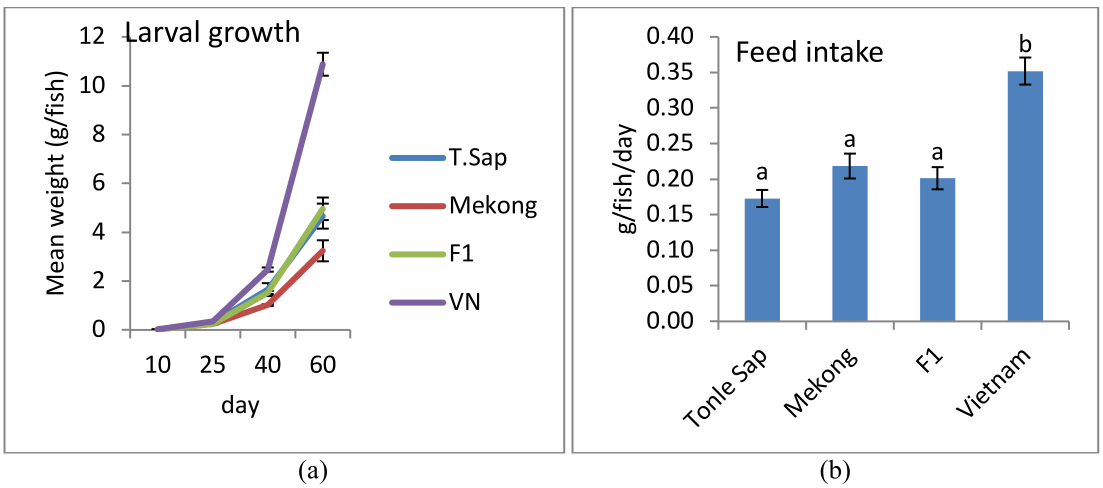


Figure1. Weight gain (panel a) and feed intake (panel b) of larvae spawned from different broodstock fish as indicated and subjected to the same weaning and rearing protocol from 10 to 60 d after hatching.

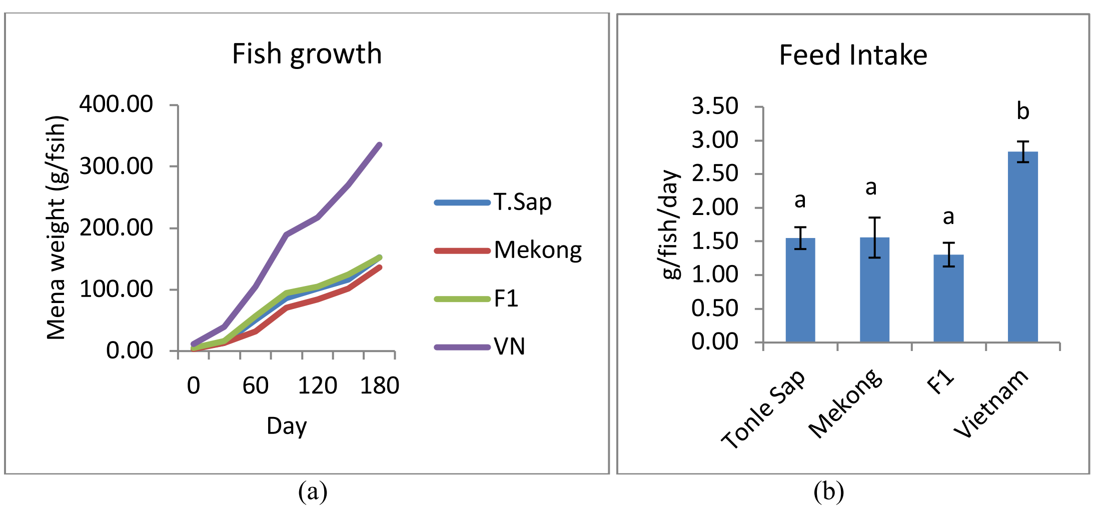


Figure2. Weight gain (panel a) and feed intake (panel b) of weaned fish from the hatchery phase (spawned from different broodstock fish as indicated) and subjected to the grow-out rearing protocol for 6 months beginning at 60 d after hatching.

Figure 3. Snakehead spawning, weaning and rearing activities in Vietnam

Figure 4. Wild snakehead collection and condition in Cambodia

Figure 5. Induced spawning of snakehead brooders in Cambodia

Figure 6. Snakehead egg collection and incubation after spawning in Cambodia

Figure 7. Weaning snakehead larvae in hapa nets and in tanks in Cambodia

Figure 8. Snakehead fingerling after 60 days of weaning in Cambodia

Figure 9. Grow-out snakehead in hapa nets for 6 months in Cambodia

TILAPIA AND KOI (CLIMBING PERCH) POLYCULTURE WITH *PANGASIUS* CATFISH IN BRACKISH (HYPOSALINE) WATERS OF SOUTHERN BANGLADESH

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND02NC

Zahid Parvez Sukhan[1], Md. Lokman Ali[2], Md. Jabir Al Mehedi[2], Md Gias Uddin[2], Shahroz Mahean Haque[3], Courtney A. Deck[4], Russell J. Borski[4]

[1]*Department of Marine Fisheries & Oceanography, Patuakhali Science & Technology University, Patuakhali, Bangladesh*
[2]*Faculty of Fisheries, Patuakhali University of Science and Technology, Patuakhali, Bangladesh*
[3]*Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, Bangladesh*
[4]*Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA*

ABSTRACT

Pangasius catfish (*Pangasius hypopthalamus*), tilapia (*Oreochromis niloticus*), and freshwater perch (*Anabas testudineus*; locally known as Koi) are three of the most successful freshwater aquaculture species in Bangladesh. We previously established that *Pangasius* can be cultured in hyposaline waters, allowing for the use of coastal waters affected by salinity encroachment. In the present study, we assessed the potential for culturing *Pangasius* with the higher valued tilapia and freshwater Koi in hyposaline waters of coastal Bangladesh as a means of improving the food security and economic viability of these salinity affected region's poor communities. Two experiments were performed to determine the feasibility of Tilapia and Koi (climbing perch) polyculture with *Pangasius* catfish in hyposaline waters. These experiments assessed: 1) production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters, and 2) the effect of stocking density on growth performance of tilapia and *Pangasius* in polyculture.

The first experiment was conducted in 12 farmer's ponds in the Patuakhali district of Bangladesh at a salinity of 5-6 ppt. Four culture types were examined with fish being fed commercial pellet feed (CP 28-30%) twice daily at a total rate of 10% down to 3% body weight per day. The study revealed that Koi can be cultured in saline waters as high as 6 ppt and that the highest yields could be obtained through solo culture of *Pangasius* (T1). However, due to the higher market value of tilapia and greater increases in body weight of both *Pangasius* and tilapia, the largest profit came from the co-culture of these two species (T2). This was followed by co-culture of *Pangasius* with Koi (T3). Conversely, the lowest yield and profit resulted from the culture of all three species together (T4). Thus, the results suggest that tilapia is the optimal candidate for polyculture with *Pangasius* in hyposaline waters with *Pangasius*-Koi polyculture providing the second greatest profit margin. Adoption of such aquaculture practices in coastal Bangladesh could increase earnings and improve the livelihoods of the people inhabiting these areas.

The second experiment was carried out to evaluate the effects of 3 different stocking densities on growth performance of *Pangasius* and tilapia (*O. niloticus*) in polyculture. In treatment 1 (T1), *Pangasius* and tilapia were stocked at a density of $2.5/m^2$ and $2/m^2$, respectively. In treatment 2 (T2), *Pangasius* and tilapia were stocked at a density of $2/m^2$ and $2.5/m^2$, respectively. Lastly in treatment 3 (T3), *Pangasius* and tilapia were stocked at a density of $1.5/m^2$ and $3/m^2$, respectively. No significant differences (P>0.05) were observed in survival rate, feed conversion ratio (FCR), or weight gain of tilapia among the treatments, however the size and overall production yields of *Pangasius* declined as the stocking density of tilapia increased. Thus, significantly higher production was observed in T1

(16,320 kg/ha), followed by T2 (21,069.05 kg/ha) and T3 (18,098.54 kg/ha). Net profit was greatest in T1 (6,751 USD /ha) than in T2 (5,798 USD /ha) and T3 (4,127 USD /ha). These results indicate that high stocking densities of tilapia are not suitable for polyculture with *Pangasius.* A higher stocking density of *Pangasius* (2.5/m^2) and lower stocking density of tilapia (2/m^2) is more suitable and economically profitable for *Pangasius* polyculture.

INTRODUCTION

The focus of this investigation was to assess the potential for expanding the culture of economically important finfish traditionally grown in fresh water to the hyposaline waters of coastal South and Southwest Bangladesh that are now significantly contaminated by saline waters. The regions are severely impacted by overfishing and with seawater encroachment and more frequent storms linked to global climate change, they are currently underutilized for fish production. Lands affected by salinity have increased from 83.3 million hectares in 1973 to 105.6 million hectares in 2009 and continue to rise rapidly (SRDI, 2010). GIS mapping shows 12 districts in Southwest Bangladesh alone are affected by salinity encroachment (Neogi, 2012). The gradual salinity intrusion in the coastal area of Bangladesh is damaging natural environments and threatening coastal biodiversity, aquaculture/agricultural production, and food security, all of which have negatively impacted the socioeconomic condition of fishers and farmers that are already among the poorest in the country.

There are several ways to mitigate the salinity problem in these areas and promote seafood production. One approach is to increase culture of marine species. However, mariculture requires considerable investment in infrastructure that is currently lacking and production of marine species is more difficult than that of freshwater fishes due, in part, to the greater complexity of larval rearing. A second approach is to promote the production of fishes that are already cultured in the country and that may tolerate hyposaline environments. The proposed research seeks to develop and establish polyculture technologies for growing *Pangasius* catfish (striped catfish, *Pangasius hypopthalamus*), Koi (climbing perch, *Anabas testudineus)*, and Nile tilapia (*Oreochromis niloticus*) in hyposaline waters endemic to Southern Bangladesh.

Tilapia (*O. niloticus*) was introduced to Bangladesh over 30 years ago and is now one of the fastest growing components of the aquaculture sector, ranking 2nd to carps in total finfish production (*Pangasius* is a close 3rd; Belton et al., 2011; DoF, 2015). While its production is primarily limited to freshwater environments, tilapia possess various characteristics that make them desirable species to culture in brackish water. They are hardy, amenable for growth in numerous culture systems, and euryhaline. They can live and readily reproduce in salinities as high as 30 ppt depending on the species or strain (for review see Suresh and Lin, 1992; El-Sayed, 2006a). Nile tilapia grow well in salinities as high as 25 ppt and evidence suggest they may grow better at 5-10 ppt than in fresh water (Payne and Collinson, 1983; Suresh and Lin, 1992). Development of their culture in saline waters has received considerable attention in Asia (Dennis et al., 2004) and the growing number of Bangladesh tilapia hatcheries and availability of seed stock readily allow for integration of tilapia into brackish water farming.

Koi culture in Bangladesh has developed considerably in recent years due to its good growth, wide acceptance, and appealing taste (Kohinoor et al., 2011; DoF, 2015). Accordingly, it now constitutes 16% of farmed fish consumed by Bangladeshis (Apu, 2014). It is a hardy, air-breathing fish capable of living in low oxygen environments (Hasan et al., 2007) and recent evidence suggests Koi larvae can be raised in low salinity waters (Nadirah et al., 2014). Additionally, in short term tank trials, Koi were shown to tolerate and grow in salinities as high as 10 ppt with little impact on growth or feed conversion (Chotipuntu and Avakul, 2010; Chowdhury et al., 2014) raising the possibility that these fish could be cultured in hyposaline waters. These studies assessed for the first time the potential of culturing Koi to market size in brackish water ponds.

Pangasius catfish was introduced to Bangladesh in the 1990's, and since then it has become a thriving aquaculture industry with over 300,000 tonnes produced annually (Ali et al., 2013; Edward and Hossain, 2010; Munir, 2009). Currently, much of the *Pangasius* production comes from the North and Central regions of Bangladesh (*e.g*., greater Mymensingh). In these regions, *Pangasius* are cultured both intensively with commercial feeds, semi-intensively (with more limited feed), and in extensive (no feed) polyculture with both tilapia and carp (Ahmed et al., 2010). High disease resistance, along with high stocking density with greater production rates (up to 120 fish /m^2, average 40 tonnes / ha; UNFAO, 2010), make *Pangasius* an ideal cultivar for increasing aquaculture production in Bangladesh, particularly in regions unfamiliar with farming this species, as well as reducing the burden of population growth. The greater Barishal district is one such region, which has traditionally relied on fishing or aquaculture of marine species (*e.g*., shrimp) for their economic livelihoods. Through over-fishing, increased shrimp disease, and the increasing frequency of natural calamities like cyclones (*e.g.* Sidr, Aila), this region is nearing depletion of wild fish stocks and currently over half a million fishermen have been suffering from severe poverty. Introducing *Pangasius* as well as tilapia and Koi cultures to these coastal communities, whose water resources are largely underutilized, could enhance the dietary consumption of protein and other nutrients for low-income families, as well as provide new sources of income and employment to the area through backward and forward linkage to the value chain. In phase I of our AquaFish Innovation Lab project, we firmly established for the first time that *Pangasius* could be cultured in hyposaline ponds with salinities as high as 12 ppt with similar growth and yield as that found in freshwater pond culture (Ali et al., 2015). Further, implementation of formulated feeds and an increase in stocking densities from 2 to 3 fish/m^2 improved profits and production yield of fishes, respectively.

Here, we expanded upon this new technology to incorporate Nile tilapia and Koi in brackish water polyculture with *Pangasius.* Both tilapia and Koi command higher prices with the market value of Koi exceeding *Pangasius* by 3 times (250-300 BDT/kg vs 80-100 BDT/kg) (Apu, 2014). Implementation of tilapia in polyculture could prove beneficial as these animals readily feed on natural productivity of ponds (plankton, plants) and on supplementary feed resulting in better utilization of resources for enhancing fish yield (Egna and Boyd, 1997; El-Sayed, 2006b). Their incorporation into *Pangasius* culture may prove more beneficial for enhancing income while also providing a more diverse crop of fishes for consumption and sale. The present research first assessed the growth and production of tilapia and Koi alone and together in polyculture with *Pangasius* in hyposaline ponds and then assessed if increasing their density could further improve fish production with the aim of enhancing the diet and economic viability of coastal communities in Southern Bangladesh.

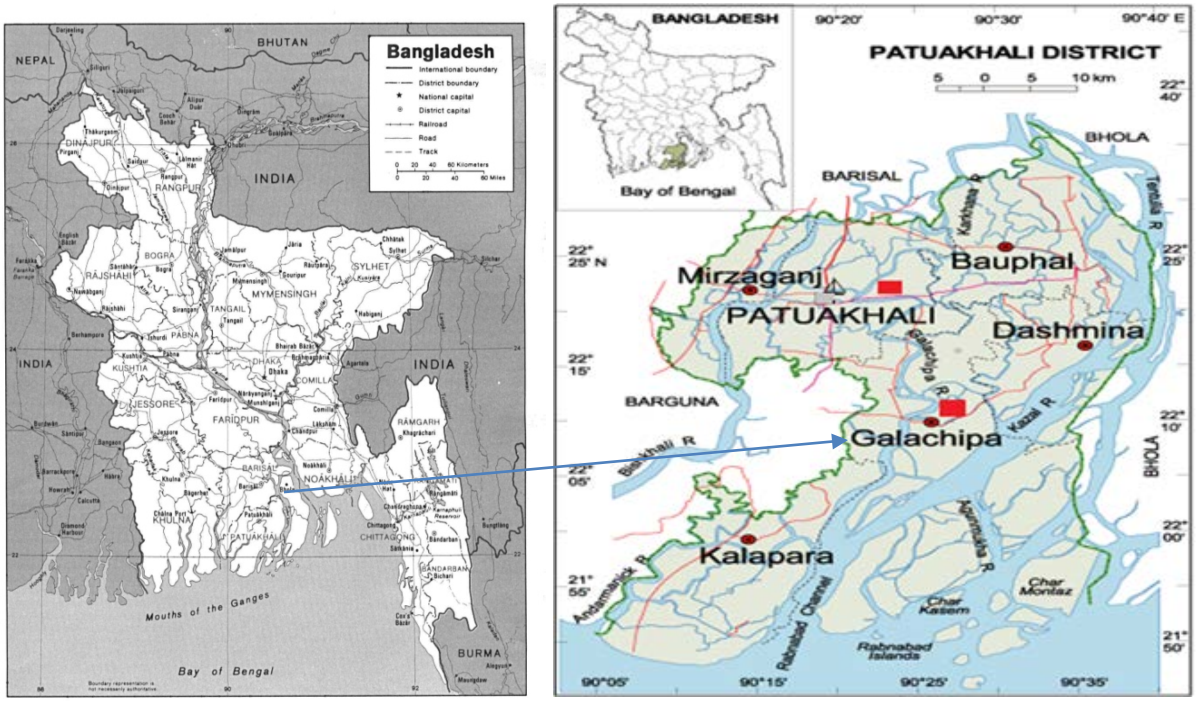
OBJECTIVES

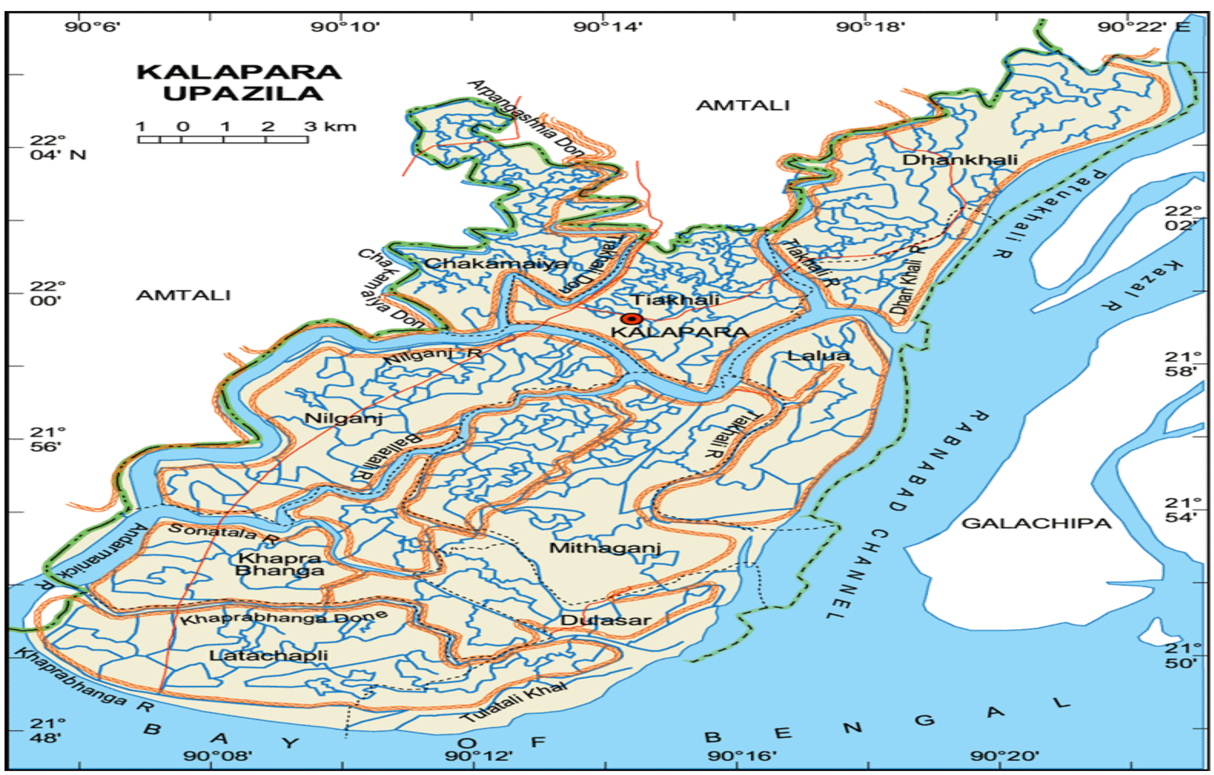
1. Evaluate if freshwater Koi (climbing perch) can be successfully cultured in seawater-encroached hyposaline waters of coastal Southern region of Bangladesh.
2. Assess production performance and economic impacts of tilapia and Koi polycultured with Pangasius in brackish waters.
3. Assess effect of increased stocking density of tilapia and Koi in brackish water Pangasius-Koi-tilapia polyculture.

MATERIALS AND METHODS

Location

Experiments were performed at Kalapara Upazila of the Patuakhali district, and area affected by salinity encroachment in Southern Bangladesh. Water quality analysis was conducted at Patuakhali Science & Technology University.





Pond Preparation

Twelve ponds (average size of 9.5 decimal, 1.2 m depth) selected from 3 areas on the basis of salinity were dried and limed (1 kg/dec $CaCO_3$) before being flooded with adjacent surface water from the Andharmanik river. Salinity was maintained at 5-8 ppt by the addition of freshwater. Rotenone was applied to ensure the complete removal of unwanted fishes and other species. All ponds were then fertilized with 150 g/dec N as urea and 75 g/dec P as triple super phosphate (TSP).

Feeding
Fish were fed a Mega floating feed (Lily Brand tilapia feed with CP 28-30%) at an initial rate of 10% bw/day down to 3% bw/day. Feed was applied twice daily in the morning and evening by hand. The amount of feed used was recorded for the calculation of feed conversion ratios (FCR).

Study 1- *Assess production performance and economic impacts of tilapia and Koi polycultured with Pangasius in brackish waters*
The study was conducted from March to July 2017 and consisted of a *Pangasius* monoculture (T1), a *Pangasius*-tilapia polyculture (T2), a *Pangasius*-Koi polyculture (T3), and a *Pangasius*-tilapia-Koi polyculture (T4). Ponds were stocked with fingerlings at the densities shown in Table 1 and each treatment was replicated in 3 separate ponds. Fingerlings were obtained from Sharnalata Hatchery at Trishal Upazilla in Mymensingh district with average initial weights of 6.5 g, 0.5 g, and 0.5 g for *Pangasius*, Nile tilapia, and Koi, respectively. Water quality parameters (*e.g.* temperature, dissolved, oxygen, salinity, pH, alkalinity, ammonia) were assessed fortnightly to ensure they remained within the range appropriate for fish culture. Ten fish from all ponds were sub-sampled fortnightly for growth measurements (length and weight) and to adjust feeding rate. At the end of the 150-day culture period, all fish were harvested to determine growth and survival and an economic (cost-benefit) analysis was performed using seasonal market prices for each species (*Pangasius*- 90 BDT/kg; tilapia- 120 BDT/kg; Koi- 250 BDT/kg).

Table 1. Experimental design for Study 1.

Parameter	*Treatment 1*	*Treatment 2*	*Treatment 3*	*Treatment 4*
Pangasius (fish/dec)	160 (4.0/m^2)	80 (2.0/m^2)	80 (2.0/m^2)	80 (2.0/m^2)
Tilapia (fish/dec)	-	80 (2.0/m^2)	-	40 (1.0/m^2)
Koi (fish/dec)	-	-	80 (2.0/m^2)	40 (1.0/m^2)
Salinity range (ppt)	5-6	5-6	5-6	5-6
Replication	3	3	3	3

Study 2- *Effect of stocking density on growth performance of tilapia and Pangasius in polyculture*
In Study 1 we determined that *Pangasius*-tilapia polyculture was most profitable and thus we evaluated which stocking densities would provide the greatest returns. The experiment was conducted from 9th October 2017 to 9th May 2018. *Pangasius* (1.36 g) and sex-reversed Nile tilapia (0.12 g) fry obtained from Sharnalata Hatchery at Trishal Upazila in Mymensingh district were stocked at the densities indicated in Table 2 and each treatment was replicated in 4 separate ponds. Water quality was assessed and ten fish from each pond were sub-sampled for growth measurements every 15 days. At the end of the 150-day culture period, all fish were harvested for growth and survival and an economic (cost-benefit) analysis was performed using seasonal market prices for each species (*Pangasius*- 120 BDT/kg; tilapia- 130 BDT/kg).

Table 2. Experimental design for Study 2.

Parameter	*Treatment 1*	*Treatment 2*	*Treatment 3*
Pangasius (fish/dec)	100 (2.5/m^2)	80 (2/m^2)	60 (1.5/m^2)
Tilapia (fish/dec)	80 (2.0/m^2)	100 (2.5/m^2)	120 (3.0/m^2)
Salinity range (ppt)	5-8	5-8	5-8
Replication	4	4	4

Equations

Survival Rate (%) = $\frac{\text{\# fish harvested}}{\text{\# fish stocked}} \times 100$

Specific Growth Rate (% bw day^{-1}) = $\frac{(\text{Log e [final weight]} - \text{Log e [intitial weight]})}{\text{Time (final)} - \text{Time (initial)}} \times 100$

Food conversion ratio = $\frac{\text{Amount of food given (dry weight)}}{\text{Fish weight gain}}$

Statistical Analysis

One-way analysis of variance (ANOVA) with a Duncan's New Multiple Range posthoc test was used to compare all water quality, growth, and production parameters between treatment groups (SPSS 11.5 software). Data are represented as means ± standard deviation.

RESULTS AND DISCUSSION

Study 1- *Assess production performance and economic impacts of tilapia and Koi polycultured with Pangasius in brackish waters*

This study evaluated whether the introduction of *Pangasius* catfish to Koi and tilapia cultures in hyposaline waters could enhance production yields and earnings for farmers in Southern Bangladesh. Water quality was evaluated throughout the experimental period. Values for water temperature, dissolved oxygen, pH, alkalinity, ammonia, hardness, nitrites, and salinity were similar between treatments and within the appropriate range for fish culture (Table 3; Bhatnagar and Devi, 2013). Feed conversion ratios were also similar between treatments and at an appropriate level for fish culture (Table 4).

Gross production (kg/ha) was significantly greater in *Pangasius* monoculture (T1) compared to the 3 types of polyculture due to the larger size of this species (Table 4). However, as tilapia and Koi fetch a greater market value and require less feed input due to their smaller size, T2 (Pangasius-tilapia) provided the greatest net return at $6260 USD/ha followed by T3 (*Pangasius*-Koi) at $4127 USD/ha (Figure 1). *Pangasius* had significantly higher specific growth rates when cultured with tilapia which could increase the amount of food available for the farmers' families (Table 4). Polyculture of all 3 species together (T4) had the lowest overall production yield and profits, possibly due to the smaller size of Koi or an antagonistic effect between species that lead to slightly lower survival rates in this treatment (Table 4). These results indicate that polyculture of *Pangasius* and tilapia is the best option for enhancing the diets and incomes of farmers whose land has been affected by salinity encroachment in Southern Bangladesh, with *Pangasius*-Koi polyculture providing the second greatest profit margin.

Study 2- *Effect of stocking density on growth performance of tilapia and Pangasius in polyculture*

After determining in Study 1 that tilapia is a better candidate for polyculture with *Pangasius*, we sought to determine the optimal stocking densities to further enhance production and earnings. Water quality was deemed suitable throughout the study period and did not vary significantly between treatments (Table 5).

Growth and production parameters are presented in Table 6. The feed conversions ratios did not vary between treatments and were within the appropriate range for fish aquaculture. Survival, growth rates, and gross production (kg/ha) of tilapia did not vary between treatments. However, the final weights and gross production of *Pangasius* was significantly greater when the stocking density of tilapia was lower. Thus, T1 exhibited the greatest overall yield (16,320 kg/ha), followed by T2 (14,318 kg/ha), and T3 (12,170 kg/ha). Despite the increased production costs resulting from the higher feed requirement of *Pangasius*, T1 also provided the greatest net return at $6,751 USD/ha (Figure 2) albeit the benefit to cost ratio was similar between T1 (1.39) and T2 (1.37), which had a net profit of $5,798 USD/ha.

The results of this experiment revealed that high stocking densities of tilapia are not suitable for polyculture with *Pangasius.* As such, polycultures employing a higher stocking density of *Pangasius* (2.5/m^2) and lower stocking density of tilapia (2/m^2) are more suitable and economically profitable for fish farmers in Southern Bangladesh.

CONCLUSIONS

This investigation revealed that Koi can be successfully cultured in hyposaline waters of up to 6 ppt. Further, we showed that Nile tilapia are the optimal candidate for polyculture with *Pangasius* catfish as this combination yielded the highest overall production and net returns. Koi also proved to be an acceptable candidate for polyculture with *Pangasius*, yielding a greater profit margin than *Pangasius* monoculture, however antagonistic effects were observed when all 3 species were cultured together. The adoption of *Pangasius* polycultures with tilapia or Koi could increase household food availability and earnings for farmers inhabiting areas affected by salinity encroachment in Southern Bangladesh.

The optimal stocking densities for *Pangasius*-tilapia polyculture were also evaluated and the results indicate that higher densities of tilapia have detrimental effects on the growth of *Pangasius*. Thus, it is recommended that these species be stocked at a ratio of 2.5 *Pangasius* to 2.0 tilapia for pond cultures using hyposaline waters.

ADDITIONAL INFORMATION

Training

Two MS graduate students were trained under this project. Additionally, as part of Investigation 16MNE02NC two short term training workshops on Pangasius, Tilapia and Koi culture in hyposaline waters were organized. Approximately 100 farmers received training on the new aquaculture production technology.

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TABLES AND FIGURES

Table 3. Average water quality parameters for Study 1. Data are means ± SD.

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Water Temperature (°C)	30.18 ± 0.95	30.10 ± 0.42	30.52 ± 0.4	30.22 ± 0.6
Dissolved Oxygen (mg/L)	5.16 ± 0.12	5.24 ± 0.13	5.23 ± 0.21	5.33 ± 0.11
pH	7.77 ± 0.37	7.49 ± 0.26	7.67 ± 0.14	7.77 ± 0.24
Alkalinity (mg/L)	90.5 ± 6.12	91.5 ± 6.61	94 ± 5.15	93 ± 5.85
Ammonia (mg/L)	0.67 ± 0.02	0.64 ± 0.03	0.71 ± 0.03	0.73 ± 0.05
Nitrites (mg/L)	0.37 ± 0.02	0.24 ± 0.23	0.29 ± 0.28	0.28 ± 0.08
Hardness (mg/L)	560 ± 4.12	540 ± 5.12	534 ± 4.92	542 ± 4.72
Salinity (ppt)	5.50 ± 0.15	5.7 ± 0.12	5.5 ± 0.13	5.9 ± 0.17

Table 4. Growth and production parameters for Study 1. Data are means ± SD. Different letters indicate significant differences (One-way ANOVA; $P < 0.05$).

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Pangasius				
Weight (g)	637 ± 6.4^a	687 ± 6.6^b	646 ± 8.1^a	672 ± 7.6^c
Survival Rate (%)	94.38 ± 2.76^a	94.58 ± 2.98^a	94.17 ± 2.12^a	92.50 ± 2.33^a
Specific Growth Rate (% bw d^{-1})	3.05 ± 0.19^a	3.11 ± 0.32^b	3.07 ± 0.23^a	3.09 ± 0.12^{ab}
Gross Production (kg/ha)	22,292 ± 141	12,837 ± 62	12,065 ± 52	6,138 ± 87
Tilapia				
Weight (g)	NA	282 ± 6.1	NA	276 ± 4.2
Survival Rate (%)	NA	90.83 ± 2.13	NA	87.5 ± 2.23
Specific Growth Rate (% bw d^{-1})	NA	4.23 ± 0.24	NA	4.21 ± 0.17
Gross Production (kg/ha)	NA	5,064 ± 123	NA	2,431 ± 12
Koi				
Weight (g)	NA	NA	169 ± 6.5	123 ± 4.9
Survival Rate (%)	NA	NA	87.92 ± 2.76	85.83 ± 2.76
Specific Growth Rate (% bw d^{-1})	NA	NA	3.89 ± 0.12	3.67 ± 0.10
Gross Production (kg/ha)	NA	NA	2,980 ± 53	1,022 ± 13
Total				
Feed Conversion Ratio	1.35 ± 0.04	1.30 ± 0.05	1.33 ± 0.08	1.33 ± 0.06
Gross Production (kg/ha)	$22{,}292 \pm 141^a$	$17{,}901 \pm 66^b$	$15{,}045 \pm 73^c$	$9{,}590 \pm 65^d$
Gross Return (USD/ha)	$23{,}884 \pm 123^a$	$23{,}441 \pm 56^a$	$21{,}726 \pm 65^b$	$13{,}089 \pm 59^c$
Cost (USD/ha)	$20{,}277 \pm 112^a$	$17{,}181 \pm 45^b$	$17{,}599 \pm 56^b$	$10{,}089 \pm 49^c$
Net Return (USD/ha)	$3{,}607 \pm 33^a$	$6{,}260 \pm 24^b$	$4{,}127 \pm 21^a$	$2{,}853 \pm 23^c$
Benefit Cost Ratio	1.17	1.36	1.23	1.29

Table 5. Water quality parameters for Study 2. Data are means ± SD.

	Treatment 1	*Treatment 2*	*Treatment 3*
Water Temperature (°C)	28.58 ± 0.25	28.34 ± 0.32	28.52 ± 0.41
Dissolved Oxygen (mg/L)	5.76 ± 0.12	5.84 ± 0.13	5.91 ± 0.21
pH	8.17 ± 0.37	8.29 ± 0.26	8.27± 0.14
Alkalinity (mg/L)	145.5 ± 6.15	140 ± 5.61	142 ± 4.15
Ammonia (mg/L)	0.27 ± 0.02	0.34 ± 0.03	0.29 ± 0.03
Hardness (mg/L)	240 ± 6.12	235 ± 7.12	242 ± 6.92
Salinity (ppt)	5.22 ± 0.62	5.28 ± 0.87	5.17 ± 0.51

Table 6. Growth and production parameters for Study 2. Data are means ± SD. Different letters indicate significant differences (One-way ANOVA; $P < 0.05$).

	Treatment 1	***Treatment 2***	***Treatment 3***
Pangasius			
Weight (g)	501 ± 68[a]	492 ± 18[b]	443 ± 44[c]
Survival Rate (%)	95.38 ± 3.16	94.58 ± 3.06	94.17 ± 3.23
Specific Growth Rate (% bw d^{-1})	3.01 ± 0.09	3.05 ± 0.11	3.07 ± 0.13
Gross Production (kg/ha)	11,806 ± 121[a]	9,193 ± 109[b]	6,184 ± 116[c]
Tilapia			
Weight (g)	233 ± 63	229 ± 22	225 ± 54
Survival Rate (%)	90.13 ± 3.16	90.83 ± 3.16	89.92 ± 3.16
Specific Growth Rate (% bw d^{-1})	4.43 ± 0.12	4.23 ± 0.14	3.89 ± 0.11
Gross Production (kg/ha)	4,514 ± 91[a]	5,124 ± 85[b]	5,986 ± 79[c]
Total			
Feed Conversion Ratio	1.35 ± 0.06	1.30 ± 0.04	1.33 ± 0.07
Gross Production (kg/ha)	16,320 ± 135[a]	14,318 ± 128[b]	12,170 ± 132[c]
Gross Return (USD/ha)	23,853 ± 132[a]	21,069 ± 114[b]	18,098 ± 127[c]
Cost (USD/ha)	17,102 ± 112[a]	15,271 ± 112[b]	13,971 ± 112[c]
Net Return (USD/ha)	6,751 ± 31[a]	5,798 ± 43[b]	4,127 ± 38[c]
Benefit Cost Ratio	1.39	1.37	1.29

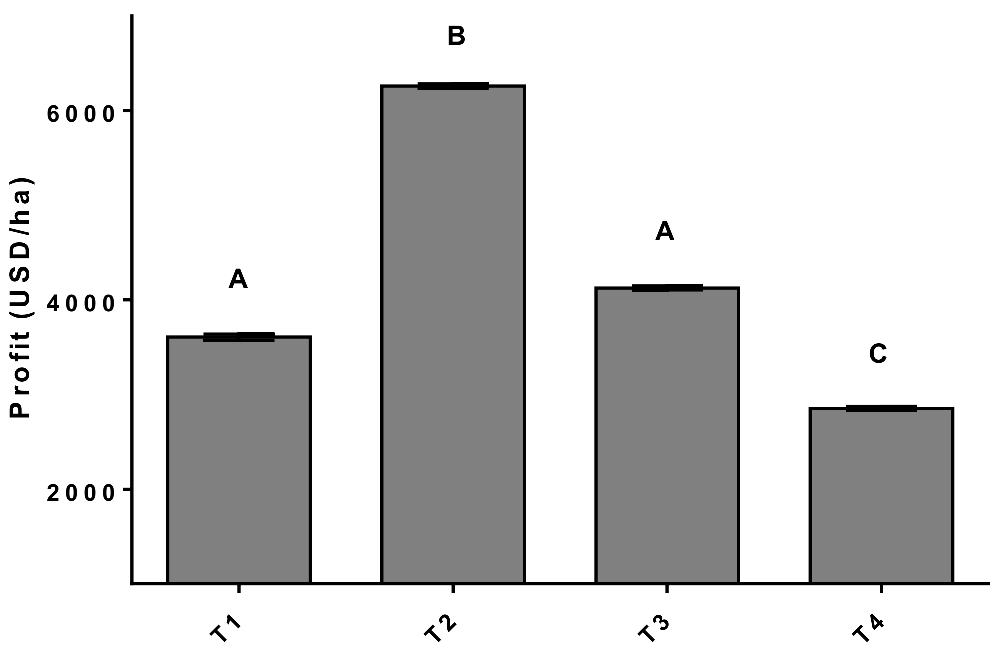

Figure 1. Net returns (USD/ha) for the culture of *Pangasius*, tilapia, and Koi in Study 1. T1, *Pangasius* monoculture; T2, *Pangasius*-tilapia polyculture; T3, *Pangasius*-Koi polyculture; T4, *Pangasius*-tilapia-Koi polyculture.

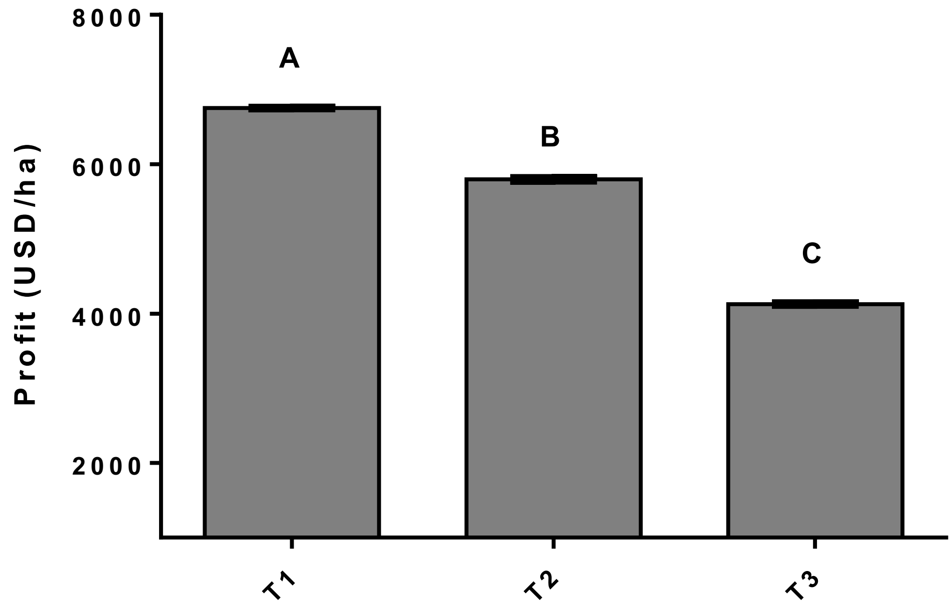

Figure 2. Net returns (USD/ha) for the culture of *Pangasius* and tilapia at different stocking densities in Study 2.

DEVELOPMENT OF CAPTIVE BREEDING, LARVAL REARING TECHNOLOGIES AND MANAGEMENT PRACTICES FOR AFRICAN LUNGFISH (*PROTOPTERUS AETHIPICUS*)

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND03AU

John Walakira[1], Benjamin Reading[2], Russell Borski[2], Jeffrey Terhune[3], Claude Boyd[3], and Joseph J. Molnar[3]

[1]*National Fisheries Resources Research Institute, Kampala, Uganda;,*
[2]*North Carolina State University, USA*
[3]*Auburn University, USA*

ABSTRACT:

African lungfish (*Protopterus aethiopicus*) supports many communities in Uganda, and has aquaculture potential in the East African region. Fish farmers access seed from natural environments, which is not sustainable, environmentally and economically. This study uncovers the genetic diversity of *P. aethiopicus* collected from Lakes Wamala, Kyoga, Nawampasa, Bisina, Edward and George with a total of putative 1,437 SNPs generated to inform its future breeding programs. Fertilized lungfish eggs will hatch at a range of 24-32C (optimally at 27C) when bred in captivity. Hatchability in captivity averaged 21.7 ± 7.2 % (SD) and mean fecundity of wild-caught broods was 1922.41 ±1227.6. A combination of salt (0.5g/L) and temperature enhance its hatchability. Larvae will start feeding artificial diets at 18-20 Day-After Hatch. Larval growth and survival is enhanced when fed on combination of decapsulated *Artemia sp.* or live *Moina and* microdiet (35-57% Crude Protein) improves growth and survival rates. This study generates information that guides the domestication of African lungfish in the region, and will improve nutrition and livelihoods of vulnerable communities.

INTRODUCTION

The African lungfish (*P. aethiopicus*) is a high value species in great demand within the lakes region of Uganda. The species natural distribution occurs in the basins of the Congo and Nile river systems in Central and Eastern Africa that include Victoria, Tanganyika, Albert, Edward, George and Kyoga lakes (Greenwood 1966). The *P. aethiopicus* was a substantial component of the lake fisheries in 1920s (Smith 1931), but because it dwells largely in swamps vulnerable to human activities, it has been overfished. Attempts to domesticate lungfish through induced spawning and evaluation of growth performance in captivity has been met with little success. Some farmers in eastern Uganda have caught young lungfish from the wild and stocked them in small ponds, but the yields have been reported to be low (Walakira 2012; 2014). We do not yet know how to reproduce the species, how best to feed the fish, or how to manage its culture and harvest in captivity.

Aquaculture requires year round supply of quality seed (Bromage et al. 2001). However, production of such seed entails development of appropriate technologies for breeding the fish in captivity and ensuring constant supply of the seed to farmers (Aruho 2013). The domestication process requires knowledge of the reproductive biology and life history of the African lungfish, which has not yet been characterized. This includes addressing questions such as spawning season, reproductive hormone profiles, egg quality and maturation, fecundity, size at sexual maturity, clear gender identification, and expected sex ratios of offspring (Bromage et al. 2001). It is equally imperative to establish larval and juvenile rearing and management protocols for successful growth of African lungfish in captivity by farmers.

This study seeks to develop sustainable breeding and appropriate culture techniques for African lungfish species using commercially available fish feeds. If feasible, culturing lungfish has the potential to improve nutrition, food security, and generate income for local farmers. It will also reduce harvest pressure on wild fish stocks in Uganda.

Successful culture techniques may offer some distinct advantages for income generation for small-scale fish farmers as a high value product grown under controlled conditions. Local people in many parts of Uganda and the East African region cherish the fish because it has a desired flesh quality, especially when fried into pieces coated with cassava, although other forms of preparation are also very common. Small ethnic groups from central Buganda region associate the lungfish with cultural beliefs and desire to preserve it.

OBJECTIVES

1. Assess the seasonal reproductive cycles of the African lungfish in two Uganda lakes.
2. Identify breeding technologies for producing African lungfish seed.
3. Determine optimal larvae weaning feed and period in *P. aethiopicus*.
4. Evaluate the culture performance of African lungfish raised from juvenile to market size in ponds and tanks.

METHODS AND MATERIALS

Experiment 1: Assess the seasonal African lungfish reproductive cycle in two Uganda Lakes.
Lake Wamala in Central Uganda and Lake Bisina in Eastern Uganda each supports established populations of lungfish. Understanding the reproductive seasonality of lungfish is necessary for identification of the right period and the right size (size at sexual maturity) of ripe broodfish to induce them to spawn in captivity. The hormonal profiles, gonad somatic indices, fecundity, size at sexual maturity, gender identification and sex ratios of the fish are subject to climatic and micro-environmental changes. Little is known about these aspects of the life history of African lungfish and they are critical to successful rearing in captivity.

Hormonal profiles and seasonality.
Monthly blood samples of more than 30 specimens (at least 15 fish from each gender) of mature *P. aethiopicus* per lake, will be collected from either the caudal peduncle or directly from the heart using a heparinised microsyringe and centrifuged at 6000 rpm for 5 minutes. Plasma was collected, frozen at -20^{o} C, and shipped to North Carolina State University for steroid assays. The steroid levels were measured using enzyme-linked immunosorbent assay (ELISA) technique following Yeo & Lim (2015).

Antisera for measuring plasma levels of 17-beta-estradiol (E2), 11-ketotestosterone (11-KT), and testosterone (T) were purchased from Cayman Chemical Company (Levavi-Sivan et al. 2004). These are the important gonadal steroids regulating gametogenesis in male and female fish and are typically used for characterizing the reproductive cycle (see: Berlinsky & Specker 1991; Jackson & Sullivan 1995).

Although we recognize that progesterones also are important mediators of gamete maturation, at this time we do not know which progesterone(s) are active in African lungfish. The progesterone maturation-inducing steroid (MIS) of fishes varies between species (Patino & Sullivan 2002) and would require characterization for meaningful interpretation of plasma levels. Therefore, measuring plasma progesterone is beyond the scope of this present application.

The seasonal variations of gonadal steroid profiles (E2, 11-KT, and T) in *P. aethiopicus* was determined by ELISA at North Carolina State University and correlated with reproductive parameters such as gonad somatic index (GSI), condition factor (K), diameter of the top clutch of oocytes, and oocyte stage (see: Wallace & Selman 1981). Analysis of variance (ANOVA) was employed to test differences in various reproductive parameters over time. The study was conducted for 12 months.

Gonad characteristics.
Somatic index (GSI), condition factor, length at maturity, fecundity and sex ratios were measured. Samples from Lakes Wamala and Bisina were collected monthly for a year, weighed and dissected to expose gonads for staging and gender identification. Standard histological methods (Bancroft and Gamble 2002) were conducted to aid confirmation of sex of gonads (especially tiny gonads) and stages. Digital photographs of the gonad histology were taken.

Gonads were staged following Brown-Peterson et al. (2011) and Wallace & Selman (1981) classification and the mean diameter of the top clutch of oocytes was measured using light microscopy. Mean differences in gonad somatic index (GSI) (Weight of the gonads/Eviscerated weight x 100), condition factor K (weight/length L^3*100) and the fecundity between and within the lakes was determined by analysis of variance (ANOVA). Fecundity was determined by gravimetric method (Kipling and Frost 1969). Significant deviation from the hypothetical 1:1 ratio of monthly and class size sex ratios were determined using a chi-square test and binomial probability.

Sexual maturity was determined by fitting a logistic ogive to the reproductive active fish captured during the spawning season in two-centimeter size classes. The logistic ogive is described by the equation $P_L = (1+\exp(L-L_{50})^{-1}$ where P_L is the percentage of the mature fish at length L and L_{50} is the length at maturity. A nonlinear minimization formula below was used to estimate the parameters.

$$-\ln L = \sum_L y_L \ln\left[\frac{P_l}{1-P_l}\right] + n_L \ln(1-P_L)$$

Where y_L is the observed numbers of mature fish in n_L, fish sampled in Length class L.

Gender identification.
Identification of males and females is a key reproductive aspect required in identifying sexes of some of the cultured fish species that cannot be easily identified as females and males. Accurate sex determination of *P. aethiopicus* is imperative to aid develop simple, user-friendly techniques for captive farming. New technological approaches have been developed to simplify identification of males and females for species that are difficult to separate. Biomarkers such as gene expression and plasma vitellogenin are used to identify gender of some fish species, however these methods rely on proper characterization of the gene expression or egg yolk systems specific to each fish species and they must be validated prior to any meaningful use (Reading et al. 2009; Reading & Sullivan 2011; Schilling et al. 2015).

Since no coding gene (RNA or cDNA) sequence is available for lungfish vitellogenin, and the species is quite divergent from other fishes with validated vitellogenin assays, measuring plasma vitellogenin is beyond the scope of the proposed project. For example, the Perciform fishes express three different forms of vitellogenin and these are quite different (structurally and functionally) from the forms of vitellogenin expressed by salmonids (Reading et al. 2009).

Little to nothing is known about the vitellogenin egg yolk systems of lungfish, and comprehensive characterization would have to precede quantification. However characterization of the yolk system of these ancient fishes would be an important future goal to eventually establishing such assays for gender identification, especially in less mature animals. Here, we propose to use expressed SNP markers and minimally invasive sampling procedures to identify mature fish of each gender.

Sample collection, morphometric, and gender identification.
African lungfish from all size classes were collected from Lakes Wamala and Bisina system and measured for total length and weight. Approximately 40-60 fish were collected using locally available harvesting gears every month. Fish were anesthetized with tricaine methane sulfonate (MS-222) buffered with 0.2 ml $NaHCO_3$, pH = 7.

Morphometric parameters were measured following the "Truss Network System" (Strauss and Bookstein 1982) focusing on the geometric morphology of the African lungfish. Each fish will have homologous anatomical landmarks. These selected *n* inter-land mark distances (modification of Cavalcanti et al. 1999) was characterized using digital images to determine the differences among wild populations of the two lakes. Data were subjected to statistical analysis as described by Mir et al. (2013) to evaluate morphological differences. Fish samples were dissected to identify individual sex, which was correlated to phenotypic observations based on existing scientific knowledge.

Ovarian biopsy method for gender identification:
A simple technique has been devised for identification of gender in other cultured fishes including the striped bass (*Morone saxatilis*) and white bass (*M. chrysops*) (Sullivan et al. 1997). In these species a short, flexible plastic cannula is inserted into the urogenital pore and a small amount of gonadal tissue is aspirated into the tube and removed for analysis under a microscope. If this tissue contains oocytes, then a positive identification of female gender can be made. If semen or no oocytes are aspirated, then identification as male is confirmed or suggested, respectively.

Ovulated African lungfish oocytes are 3.4-3.5 mm in diameter, therefore a 12 cm long cannula of about 3 to 4 mm internal diameter was used to aspirate gonadal tissue. Fish were anesthetized and oocyte staging of biopsied females was conducted as described above. Additionally, digital photographs of the oocyte stages were compiled with images collected in **1.2 Gonad characteristics** (above) to form a guide for African lungfish ovarian development.

SNP panel for gender identification.
Molecular genetic markers were identified to ascertain and explain sex differentiation and determination of African lungfish since this information is apparently unknown. Validation was measured taken to understand their effects on fish sex determination and differentiation following the Baroiller et al. (2009) method. This information will facilitate sexing the African lungfish; a basic procedure in captive breeding programs.

Through a non-lethal sampling approach, fin clips from identified fish were used to extract genomic DNA and RNA, and stored at 4 °C in 100 % ethanol and -20 °C RNA Later solution respectively. Fin clips from 40 lungfish samples (20 per site) collected from Lakes Bisina and Wamala. Expressed SNPs or Quantitative Trait Locus (QTL) be used to guide gender identification in future breeding for African lungfish.

Total RNA was extracted using a Trizol protocol and stored at -80°C. Libraries for RNA-seq were prepared from total RNA using the TruSeq manual. This study will use the Next Generation Sequencing (NGS) technology to develop a novel SNP panel that can be used for gender identification of *P. aethiopicus*. Using the reference genome and *de novo* assembly putative reads and subsequently SNPs was identified using Trinity/v2.0.6 software. The total number of SNPs detected was selected based on the SNPs expected heterozygosity and the Polymorphic information content (PIC) as a tool for genetic diversity.

DNA was prepared using protocols described by Sambrook and Russell (2001). DNA was extracted using proteinase K and phenol-chloroform and amplified using polymerase chain reaction (PCR) primers. A complete mtDNA sequence of P. *dolloi* was used to develop primers as described by Zardoya and Meyer (1996).

Following the Peukert et al. 2013 approach, genomic polymerase chain reaction (PCR)-amplification was performed in 25 µl volume of PCR buffer (0.01 M Tris, 0.05 M KCl, 1.5 mM MgCl2, 0.01% gelatin) and contained 100 ng of genomic DNA, 0.2 mM of dCTP, dGTP, dTTP, dATP, 0.2 µM of each primer and 1 U of Taq polymerase. After 3 min at 94°C, 45 cycles were made with 1 min at 94°C, 1 min at 55°C, 2 min at 72°C and a final extension step of 10 min at 72°C. Successfully amplified gene fragments obtained for reference genotypes was re-sequenced. Positions of 3'- and 5'-UTR, introns and exons was determined using GeneSeqer.

Using Ansmann et al. (2012) methods, 20 males and 20 females whose sex is phenotypically determined was verified using the SRY/ DMRT1 genes. Fragments of SRY/ DMRT1 genes was amplified using PCR with 20–25 ng DNA, 0.15 M of each primer. The PCR profile was denatured at 95°C for 4°C then cycled 35 times at 94 °C for 45 s, 50 °C for 45 s and 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products was separated on agarose gel to determine sex based on length differences

Experiment 2: Identify breeding technologies for producing African lungfish seed.
Domesticate the African lungfish using simple captive breeding techniques:
To ensure an environmentally sustainable supply of African lungfish seed to fish farmers, artificial breeding and hatching technologies was developed. Simple and low-cost breeding technologies were needed in rural communities that are dependent on this fish. Based on the described information from studies 1 and 2, mature brood-stock from the wild populations were subjected to simple artificial reproduction techniques to evaluate the working fecundity, egg survival and hatchability.
Larval rearing was conducted to determine the survival and growth aimed at establishing the larval rearing protocols (technologies) of *P. aethiopicus* to be used by the farmers. An effort also was made to retain some of these offspring in captivity to initiate a breeding program for domestic lungfish. Even a modest number of generations (i.e., 3 to 5) of breeding in captivity can greatly improve growth, tolerance to handling and poor water quality, and success at captive breeding (Teletchea & Fontaine 2014).

Identify efficient artificial breeding technologies for African lungfish:
Modifying protocols used by Vijaykumar et al. (1998), mature broods were conditioned for 3-4 weeks in concrete tanks at NaFIRRI facilities, and were induced to spawn using selected synthetic and natural hormones as described. The hormonal use will take into account the fact that *P. aethiopicus* is an asynchronous batch spawner; it releases eggs in batches. Hormones to be used will included the administration of: GnRHA, HCG, and catfish pituitary extracts.

Gonadotropin releasing hormone analogue (GnRHa) implants (added)
This is a technique that has been used to induce ovulation in cultured fish including southern flounder (*Paralichthys lethostigma*) (Berlinsky et al. 1996), wild-caught summer flounder (*P. dentatus*) (Berlinsky et al. 1997), and striped bass (Hodson & Sullivan 1993; Sullivan et al. 1997). A GnRHa-loaded implant was applied at two doses: 10 µg kg^{-1} BW (IMP-10, 6 females and 5 males); or 50 µg kg-1 BW (IMP-50, 6 females and 5 males).

Human chorionic gonadotropin (HCG) and LHRHa
An application rate of 2.0 mL per 5 kg fish HCG (330 IU/kg) and LHRHa (300*u*g/Kg) to ripe male and female broods was used, following Hodson and Sullivan (1993) protocols.

Catfish pituitary extracts (African catfish, Clarius gariepinus).
These are widely used to induce most cultured species in Uganda at a dosage of 0.014g ml^{-1}. The analysis examined fecundity, hatchability and survival of post-hatchlings. Water quality parameters were monitored weekly to understand environmental factors affecting captive breeding. Best approaches were selected based on statistical analysis of factors that produce better quantity, viability, and quality of lungfish spawns.

Compare breeding methods of African lungfish in captivity:
Manipulated environment breeding.
Selected mature broodfish (males and females) were stocked in concrete tanks or hapas suspended in earthen ponds, and then covered with macrophytes such as water hyacinth (*Eichornia crassipes*) or other aquatic plants that are usually present in natural breeding habitats. Water levels were manipulated to simulate a flood pulse to promote natural ovulation, spawning, and fertilization. Fecundity, hatchability and survival of post-hatchlings was evaluated.

Evaluating hormone induced fish.
Two approaches were used. First, the fish was allowed to spawn volitionally in ponds or tanks. Second, stocking the mature fish without hormone induction in the prepared ponds or tanks to mimic the natural environment.

Cross tabulation methods were used to analyze the egg and larval mortalities and differences in means was evaluated by analysis of variance (ANOVA) or non-parametric analog tests. Water quality parameters—pH, alkalinity, temperature—were monitored weekly to understand environmental factors affecting artificial breeding. The two approaches were evaluated based on the relative quantity, viability, and quality of lungfish spawn produced.

Experiment 3: Determine optimal larvae weaning feed and period in *P. aethiopicus.*
In larval development identification of better feed must be synchronized with the development of the weaning protocol. The optimal feed and the period for weaning are critical factors for improving the larval survival and growth for seed production (Mai et al. 2005). Weaning will enable introduction of dry feeds at a particular time and stage when the fish can easily digest and absorb the feeds. The introduction of micro diets to the developing larvae is an important strategy that is cost effective in minimizing expenses of preparation of live feeds or artemia (Gordon et al. 2000).

There is currently no weaning protocol farmers could use to produce the required larval weaning technologies (protocols). Weaning was tailored towards identifying when it is best to introduce the micro dry diets to improve the survival of larvae as well as juveniles. In this study a local commercial micro diet Ugachick (U) and Sabra & Sons (30, 35 & 45% crude protein) and live feed (de-cysted artemia and moina), and combination was evaluated for their performance on survival and growth of the larvae. Larvae from induced spawning experiments was randomly allotted to 20 tanks each of 50 liters.

Five larval feeds (including one with a combination of live and dry feed and a dry feed alone) was given to the larvae in 4 replicates. The larvae after hatch was raised until the juvenile stage. Sampling was done at intervals to record the weights and lengths of the fish. The analysis of variance (ANOVA) was used determine any differences in growth parameters such as weight gain, condition factor, specific growth rates and survival rates between various feed treatments. Duncan's test was used to establish significant variations between feeds at 95% confidence levels.

Experiment 4: Evaluate growth performance of African lungfish raised from juvenile to market size in different culture systems.

Fish development occurs in stages and each developmental stage may require feeds with varying nutrient requirements within the same species (De Silva and Anderson 1995). Juvenile lungfish were randomly allocated into six tanks and a commercial feed of varying crude protein content (35% and 40%) was evaluated to establish its performance on the growth of *P. aethiopicus*. The same experimental arrangement was evaluated in ponds at the research station and with some farmers (on farm).

Fish were sampled monthly to collect their weights and the lengths until an acceptable market size. The differences in mean weight gain, feed conversion ratios, specific growth rates and survival rates were determined by an ANOVA. The experiment was conducted for 120 days. All the fish were harvested, counted and weighed individually. The increase in length and weight was calculated from: final - initial length or weight. Survival rate was calculated on the basis of the number of fish harvested from the formula:

$$\text{Survival rate} = \frac{\text{No of fish harvested}}{\text{No of fish stocked}} \quad X\ 100\%$$

- Gross production was determined from the expression;
- Gross production = (Average final weight of fish X Total no of harvested fish)
- Net production was determined from the expression;
- Net production = (Average final weight increase X Total no of harvested fish) kg

Specific growth rate (SGR) was calculated with the formula:

$$SGR = \frac{[\ln(Wt) - \ln(Wi)] * 100}{t}$$

- InWt = natural log of the weight of the fingerlings at harvest
- InWi = natural log of the weight of the 21 days old stocked larvae.
  t = the nursing period, in days
- SGR is multiplied by 100 to express it in percentage per day

Statistical analyses use ANOVA to determine differences between the means of growth, survival and production of different treatments taking at 1 and 5 percent significance levels using the computer program.

RESULTS

Experiment 1: Assess the seasonal reproductive cycle of the African lungfish in two Uganda Lakes.

Seasonal reproductive cycle in Lakes Bisina and Wamala, Uganda Lakes.

Preliminary results from 962 feral lungfish samples indicate that this fish was continuously breeding in Lake Wamala during the seven sampling months.

Hormonal profiles and seasonality

A total of 767 blood samples were drawn from wild-caught live lungfish harvested from Lakes Bisina and Wamala, and stored under -80^{0}C during May, June, July, August, September, October and November 2017. A total of 300 well-preserved blood samples were shipped to North Carolina State University Laboratories for steroid profiles.

Gonad somatic index GSI and seasonality

The GSI was not significantly different among months both in females ($F_{6\text{-}330}$ = 1.7, p = 0.099) and males ($F_{6\text{-}115}$ = 0.0972, p=0.448) from Lake Wamala. The highest peak for females was from May –

July and from males was August and September. Results from Bisina show that significant differences occurred among the female monthly GSI ($F_{6\text{-}295} = 2.775$, $p = 0.013$). The highest peaks were recorded in the month of August, September and November. However there were no significant differences observed among the monthly Male GSI ($F_{6\text{-}209} = 1.497$, $p = 0.18$) the highest mean GSI for males was observed in July and November.

Gender identification

Ovarian biopsy method for gender identification:

A simple technique has been devised for identification of gender in other cultured fishes including the striped bass (*Morone saxatilis*) and white bass (*M. chrysops*) (Sullivan et al. 1997). In these species a short, flexible plastic cannula is inserted into the urogenital pore and a small amount of gonadal tissue is aspirated into the tube and removed for analysis under a microscope (Figure 1). If this tissue contains oocytes, then a positive identification of female gender can be made. If semen or no oocytes are aspirated, then identification as male is confirmed or suggested, respectively.

Ovulated African lungfish oocytes are 3.4-3.5 mm in diameter; therefore a 12 cm long cannula of about 3 to 4 mm internal diameter can be used to aspirate gonadal tissue. Fish were anesthetized and oocyte staging of biopsied females were conducted as described above. Additionally, digital photographs of the oocyte stages were compiled with images collected in 1.2 Gonad characteristics (above) to form a guide for African lungfish ovarian development**.**

The plastic catheter can only be inserted into the urogenital pore as far as the oviduct is straight. Maturing oocytes in the anterior part of the ovary pass through the anterior loop and then descend posteriorly toward the urogenital pore through the coiled oviduct. The African lungfish has an asynchronous ovary and will ovulate a small clutch (200-500) oocytes at a time that will travel down the oviduct for spawning. Females are iteroparous and may spawn multiple times during the breeding season.

The ovary biopsy technique has been used to sample small amounts of tissue from the gonads of several different species of fishes, including the striped bass (*Morone saxatilis*) and white bass (*M. chrysops*) (Sullivan et al. 1997). The gonads in these species are connected to the urogenital pore by a straight duct that extends from the posterior end of the organ to the exterior of the fish (urogenital pore), thereby allowing direct entry of the plastic cannula into the gonad. In the case of the African lungfish, the plastic cannula can be inserted several cm, however no gonadal tissues may be aspirated (Figure 1; see also Figure 2).

General gross anatomy of the African lungfish abdominal cavity

An overall outline of the fish is presented in Figure 3. Upon dissection, it appears that the lungfish has an unusual paired ovary compared to that of teleosts in that the oviduct originates from the anterior portion of the organ and then transverses posterior to the urogenital pore (Figures 4 and 5). The oviduct appears to be coiled and therefore the cannula cannot be fully inserted for gonadal tissue sampling from the ovarian lamellae (Figure 2). It may be that spawning lungfish with ovulated eggs in the oviduct could be sampled in this manner, however this has not been tested as none of the stage 3 females had ovulated eggs contained within the duct at the time of capture.

Batch Fecundity

A total of 81 females have been collected from Lake Wamala, preserved and later counted. The preliminary results indicate that the relationship between length and fecundity is weakly associate ($R^2 = 0.161$; Figure 3). The Number of eggs per g was 0.8±0.4, which was low. For the 26 females collected from Lake Bisina the relationship between fecundity and length was moderately associated (R^2 = 0.635). The number of eggs per gram was 0.9±0.5.

SNP panel for gender identification

Comparative genomics revealed 1285 orthologous genes that are common to the species from six lakes. Orthologous genes are unique to each lake (Bisina, 186; Edward, 138; George, 304; Kyoga, 205; Nawampasa, 250; and Wamala, 91), respectively. We also report a set of SNP markers developed by sequencing the study.

A total of 5961 SNPs sites were identified from 71191331 Illumina MiSeq reads. Of these, a total of 277 markers were selected for use in low cost typing using PCR-RFLP and can be used for monitoring the genetic diversity during conservation and management program of the wild stocks of lungfish. Besides, the genomic resources generated in this research can propel further studies of this native species.

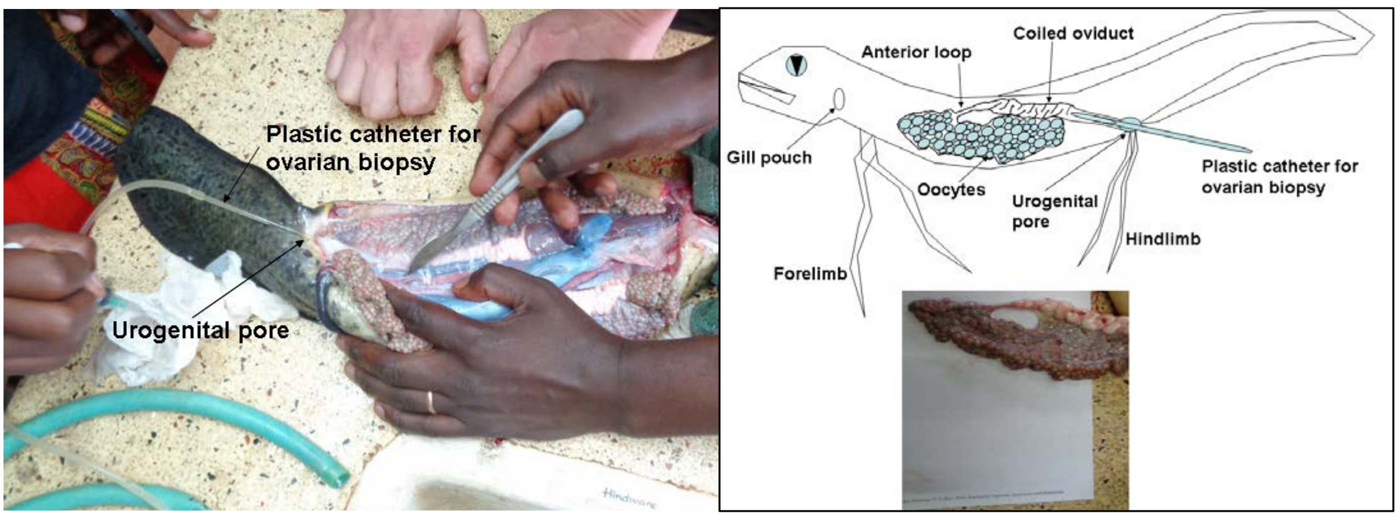

Figure 1. Photo (above) and drawing (below) of the African lungfish ovary anatomy. A plastic catheter has been inserted several centimeters into the oviduct of the African lungfish (*Protopterus aethiopicus*) and the tip of the scalpel indicates where the duct begins coiling. Oocytes pass down the oviduct from anterior to posterior. Due to the anterior loop leading from the ovary proper, it is not possible to sample oocytes directly from the ovary using the biopsy tool unless the oocytes have been ovulated and are present in the posterior oviduct near the urogenital pore.

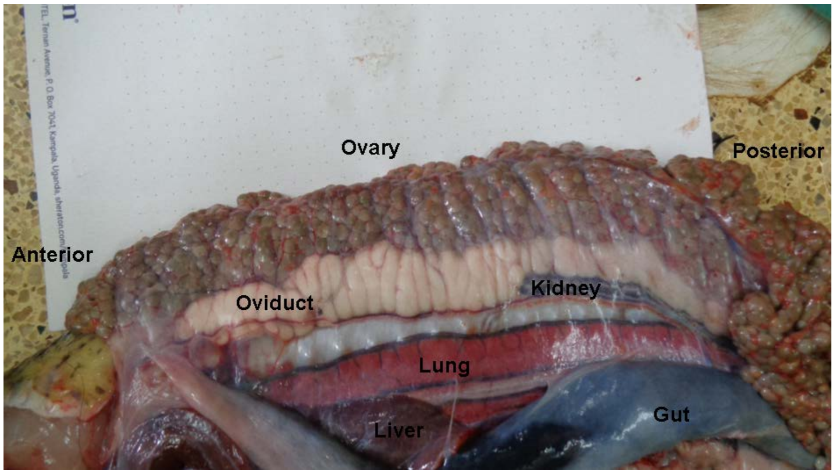

Figure 1 African lungfish (*Protopterus aethiopicus*) gross abdominal dissection showing the olive colored oocytes within the ovary, the white, coiled oviduct and associated kidney, the lung, the liver, and the gut (alimentary canal).

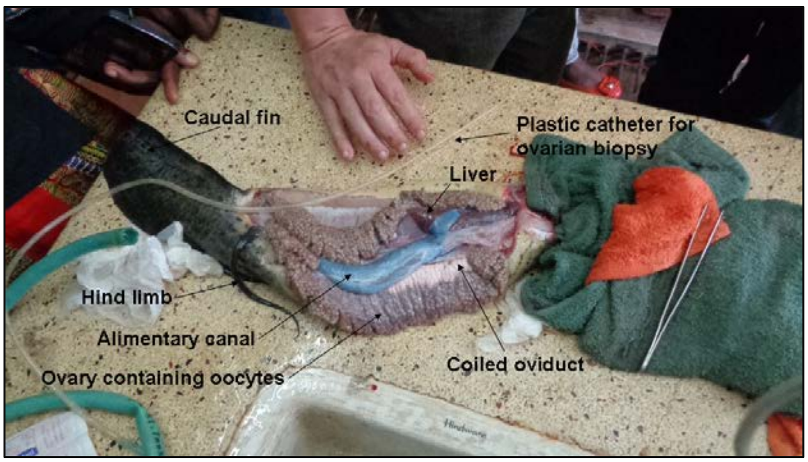

Figure 3 African lungfish (*Protopterus aethiopicus*). African lungfish (*Protopterus aethiopicus*) ovary showing the olive colored oocytes, with the largest clutch being about 3.5 mm in diameter. A short anterior loop connects the ovarian sac containing the oocytes to a coiled oviduct that extends posterior to the urogenital pore. Scale bar = 10 mm

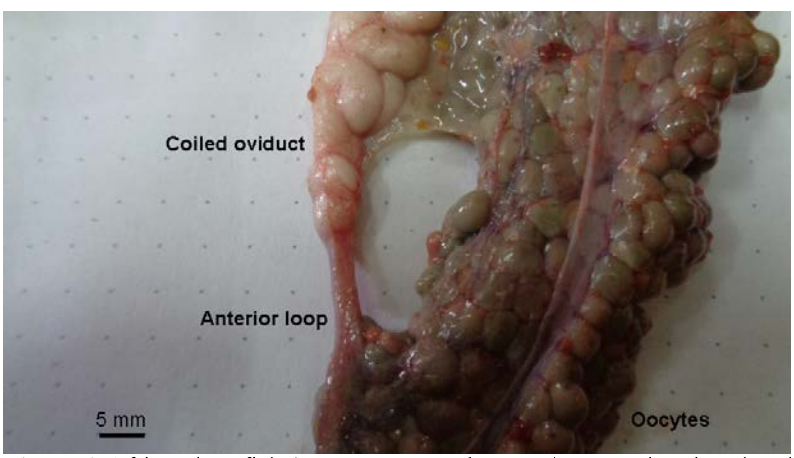

Figure 2 African lungfish (*Protopterus aethiopicus*) ovary showing the olive colored oocytes, with the largest clutch being about 3.5-4.5 mm in diameter. A short anterior loop connects the ovarian sac containing the oocytes to a coiled oviduct that extends posterior to the urogenital pore. Scale bar = 5 mm.

Experiment 2: Identify breeding technologies for producing African lungfish seed.
Domesticate the African lungfish using simple captive breeding techniques:
To ensure an environmentally sustainable supply of African lungfish seed to fish farmers, artificial breeding and hatching technologies was developed. Simple and low-cost breeding technologies were needed in rural communities that are dependent on this fish. Based on the described information from studies 1 and 2, mature brood-stock from the wild populations were subjected to simple artificial reproduction techniques to evaluate the working fecundity, egg survival and hatchability.

Evaluating hormone induced fish
HCG and LRHA analogs
Lungfish (200-300g) responded to doses of HCG and LHRH synthetic hormones but LHRHa had more effect for lungfish to spawn (Table 1). However, most induced broods failed to breed naturally under captive conditions in out-door tanks. Stripping and fertilization was possible with a few ripe wild-caught lungfish fish in hatchery conditions.

Table 1. Induce response of Lungfish broods when subjected to HCG and LHRHa doses.

HORMONE USED	MASS (g)	40% VOLUME/mls 0 hrs	60% VOLUME/mls 24-hours	TOTAL VOLUME/mls	RESPONSE
LHRHa	187	0.24	0.36	0.6	None
(300*u*g/Kg)	221	0.24	0.36	0.6	None
	361	0.32	0.48	0.8	Active
	354	0.32	0.48	0.8	Active
	480	0.4	0.6	1.0	Active
	460	0.4	0.6	1.0	Active
HCG	172	0.24	0.36	0.6	None
(400 IU/Kg)	153	0.24	0.36	0.6	None
	184	0.24	0.36	0.6	None
	258	0.24	0.36	0.6	None
	354	0.32	0.48	0.8	Active
	344	0.32	0.48	0.8	Active

Males
Male lungfish (av. 280g; 43.2cm) subjected to HCG produced milt during stripping. Only one male fish was observed to have motile milt (spermatids) when observed histologically through microscope. Spermatogonia in males dominated immature stages. The primary spermatogonia Sg1 (41± 5.3) developed into secondary spermatogia Sg2 (26.2 ± 3). While the secondary spermatogonia were transformed into primary spermatocytes Sc1 (14 ±1.3) which later developed into secondary spermatocytes Sc2 (9.76 ±1.3). The presence of Sc1 and Sc2 in the testes indicated the beginning of the developing phase. The Sc2 develop into the spermatids St (6.7±1.1) which are encysted in a coat like integument.
The size of the spermatogenic cells progressively reduced from the Sg1 to the spermatids and their color was more basophilic with Hematoxylin and Eosine (H&E). The spermatids finally burst open giving rise to the spermatozoa Sp (4.5±0.6) which are characterized by the presence of the head and the tail (Table 2 and Figure 5). The spermatids and the spermatozoa in the testes indicated stage 3 the capable spawning stage.

Table 2. Lungfish macroscopic and microscopic description of male spermatogenic cell stages.

Spermatogeic cell types	Size µm
Primary spermatogonia	41± 5.3
Secondary spermatogonia	26.2 ± 3
Primary spermatocytes	14 ±1.3
Secondary spermatocytes	9.76 ±1.3
Spermatids	6.7±1.1
Spermatozoa	4.5±0.6

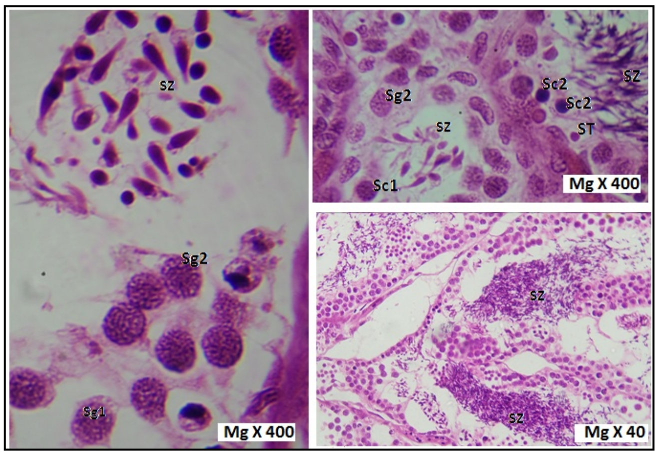

Figure 3. H & E section through lungfish testes showing a) primary spermatogonia (Sg1) b) secondary spermatogonia (Sg2) c) primary spermatocytes (Sc1) d) secondary spermatocytes (Sc2) d) spermatozoan (SZ) and e) spermatids (ST).

Females

One female released a few eggs (61) when stripped while other eggs of suspected ripe females were extracted. Microscopic examination indicated eggs at differential stages (I - IV) of maturity. Most eggs had nucleus not positioned at the animal pole, hence were not ready for ovulation and fertilization.

The ovaries of immature fish are dominated by the oogonia OO and Primary growth oocytes PGO. The oogonia were smallest germ cells with no distinct boundaries found solitary or in clusters of lamellae with a large light nucleus. The oogonia (347.44 ± 50µm) developed into primary growths oocytes (670.6 ±153.05 µm) (Figure 6). The PGO developed into cortical alveolar oocytes CAO which later transformed into the primary vitellogenic oocytes Vtg 1.

The Vtg1 developed into the secondary vitelogenic oocytes (Vtg2). The presence of CAO (1381.7±114.6µm) marked the beginning of the developing phase which also could include the Vtg1 (1400.1±190.9µm) and Vtg2 (1795.8±280.7µm) (Figure 7). The Vtg2 grew into the tertiary vitellogenic oocytes Vtg3 (2472.48 ± 284.8). The Vtg3 developed into mature oocytes MO (3737.3 ± 371.4). The Vtg3 and the MO were differentiated by the location of the nucleus. The Vtg3 had the nucleus in the center and the MO had the nucleus towards or at the periphery (Figures 7). The mean diameter of un-ovulated mature oocytes in females varied from 347.44 ± 50µm to 3737.3 ± 371.4µm (Table 3).

Table 3. Lungfish macroscopic and microscopic description of female cell stages.

Ovarian cells	Size (μm)
Oogonia	347.44 ± 50
Primary growth oocytes	670.6 ± 153.05
Cortical alveolar oocytes	1381.7 ± 114.6
Primary vitellogenic oocytes	1400.1 ± 190.9
Secondary vitellogenic oocytes	1795.8 ± 280.7
Tertiary vitellogenic oocytes	2472.48 ± 284.8
Mature oocytes	3737.3 ± 371.4

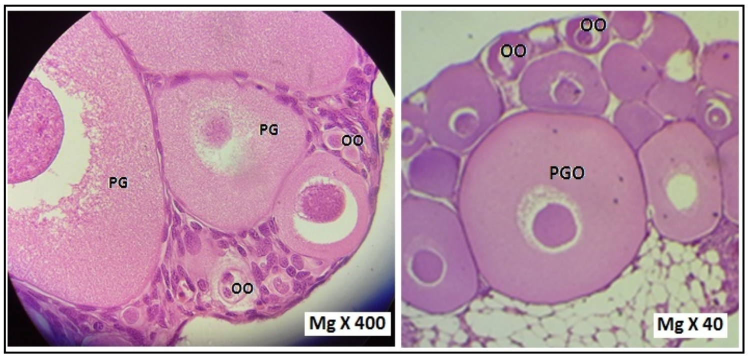

Figure 4. H & E Sections through the ovary of stage I showing oogonium (OO) and primary growth oocytes (PGO).

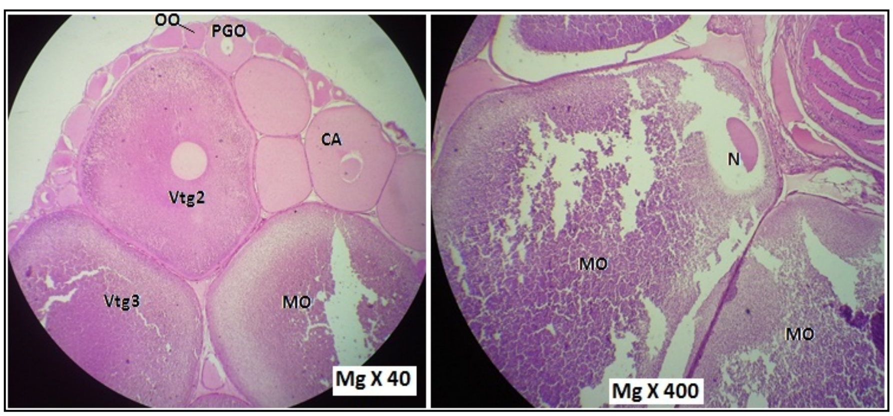

Figure 5. H & E sections through the ovary of stage III showing mature oocytes (MO) with nucleus (N) at animal pole, secondary vitellogenic oocytes (Vtg2), Tertiary vitellogenic oocytes (Vtg3) and cortical alveolar oocytes (CA).

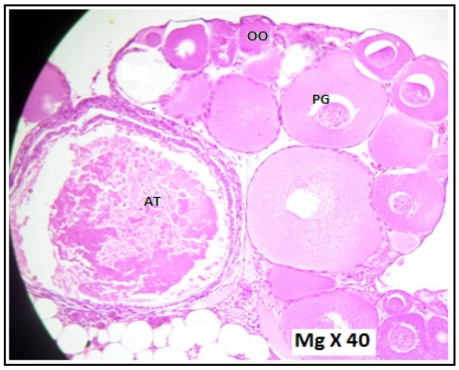

Figure 6. H & E section through the ovary of stage VI showing atretic oocytes (AO).

Hatchability

There was no significant difference ($p > 0.05$) in hatchability of lungfish eggs when exposed to temperatures of 24, 27, 30 and 32^0C (Fig. 9 ,10 and 11). Hatchability was faster at 32^0C with optimal range of 24 - 32^0C ($F_{3, 11}$=1.429; p=0.3.04 but overall rate was relatively low 9 to 14%. The hatchability was generally very low across all temperature treatments with the highest mean of 12.6 at 32^0C (Figure 11). Eggs in tanks containing water at 32^0C hatched first at 1 Day After Hatch (DAH) followed by eggs at 30^0C, 27^0C and lastly 24^0C. Tanks at 30 and 32^0C also developed fungal infections (Figure 12) which happened on the second day of hatching but more prevalent in 30^0C.

At hatching, the larvae attained the color of the eggs from which it hatched and they became uniform at the third day after hatch (Figure 13). After washing the eggs in the salt solution, it was seen that the mobility of the newly hatched larvae increased. For the eggs that delayed to hatch, in most cases the hatched larvae were weak and could die within 3 days.

GnRHa implants

There wasn't any response to implants since no lungfish hatchlings were observed in the out-door tanks.

Catfish pituitary extracts (African catfish, Clarius gariepinus).

There was no response to pituitary extracts on wild caught lungfish broods.

Manipulated environment breeding.

Wild caught lungfish broods did not breed in the out-door tanks. No hatchlings were observed.

Figure 7 Fertilized lungfish eggs (3-5 mm) hatching.

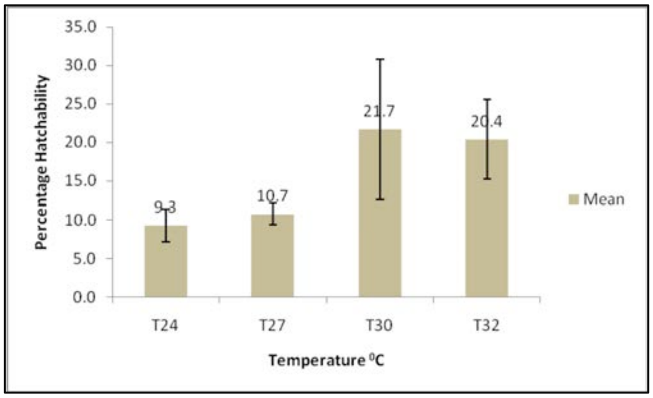

Figure 8. Hatchability rates of lungfish.

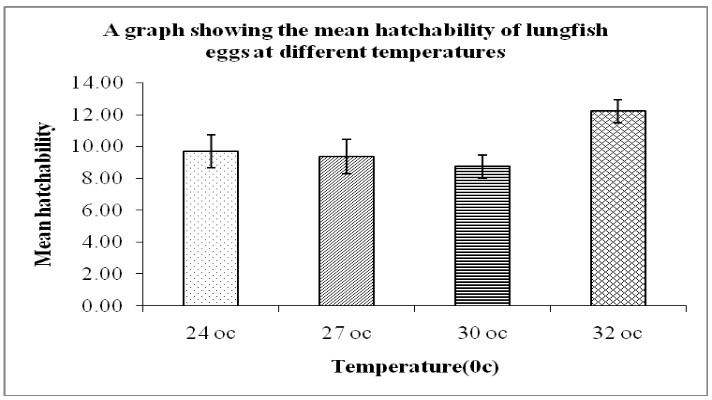

Figure 9. Mean hatchability of lungfish eggs at different temperatures

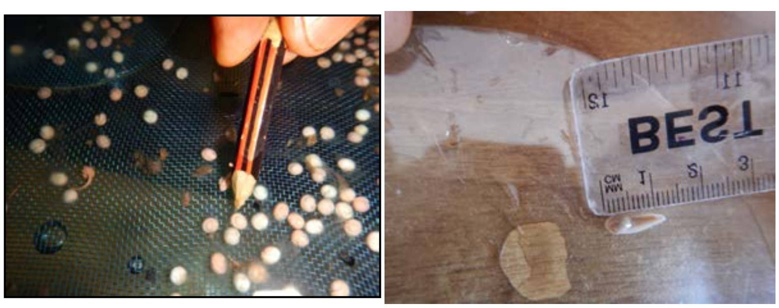

Figure 10. Lungfish eggs hatching to brown and light green larvae (av. 1.2 cm in length). White colored eggs did not hatch

Figure 11 Lungfish hatchlings/larvae in aquarium tank.

Optimal conditions for nursing lungfish hatchlings
Weaning lungfish larvae on decarpsulated Artemia, dry feed (56% CP) and their mixture showed results. However, significant differences between feed treatments ($P < 0.05$) with larvae fed decapsulated *Artemia* providing the best growth, followed by a combination of dry feed and *Artemia*; and the least being dry feed alone (Figure 14). Survival was high in treatments that were given decapsulated *Artemia* followed by the combination diet and lastly the dry feed.

Temperature:
There was no significant difference in larval growth across all water temperatures from 5 to 20-DAH. However, at 25 -DAH (27^{0}C) larvae grew significantly larger than those in 32^{0}C (Figure 9). No significant difference ($p > 0.05$). observed in weight gain with larvae raised at 24, 30 and 32^{0}C (Figure 15, 16, 17 &18). At 3 -DAH larvae at 30 and 32^{0}C changed color from brown to black while those at 27 and 24^{0}C turned black when at 4 to 6 DAH. On day four after hatch, blood vessels were visible around the belly.

Larvae in 30 and 32^{0}C started swimming in schools on 8-DAH and those at 24 to 27^{0}c, started swimming at 11 DAH. At 5-DAH, all larvae had almost the same weight though that of larvae in 30^{0}C was slightly higher.

Weaning
Histological sections revealed that development of larval digestive system was a slow transformation with complete development and differentiation of organs (buco-pharynx, oesophagus, intestine, liver and pancreas) complete by 26 DAH. At 5 DAH, the larvae changes color from grey to dark. Artemia was observed in the gut at 18 DAH. Mature columnar cells were observed at Dph 26. At the onset endogenous feeding (5-DAH when yolk reserves were not completely depleted but exhausted at 20 DAH. At this time the gut achieved its complete differentiation from the yolk sac. The mouth is terminal and protrusible, with vomerine teeth and taste buds. The digestive tract is also lined by a stratified squamous epithelium (Figure 16).

Another study was also conducted to determine whether live feed (*Artemia urmiana*), commercially prepared diet and a combination of the two feeds would enhance the growth and survival of lungfish hatchlings. Hence, triplicate of treatments: (1) dry feed (56% CP), (2) decysted artemia and (3) combination of decysted artemia (50% of food) were evaluated. After Six weeks of growth, the final weight (0.22± 0.01g) of fry fed on artemia alone was significantly higher ($p=0.000$) than fry fed on (1) and (3); (Figure 18). Survivals were similar throughout all treatments ($p=0.111$) and water quality parameters were within the recommended ranges and did not differ significantly ($p > 0.05$).

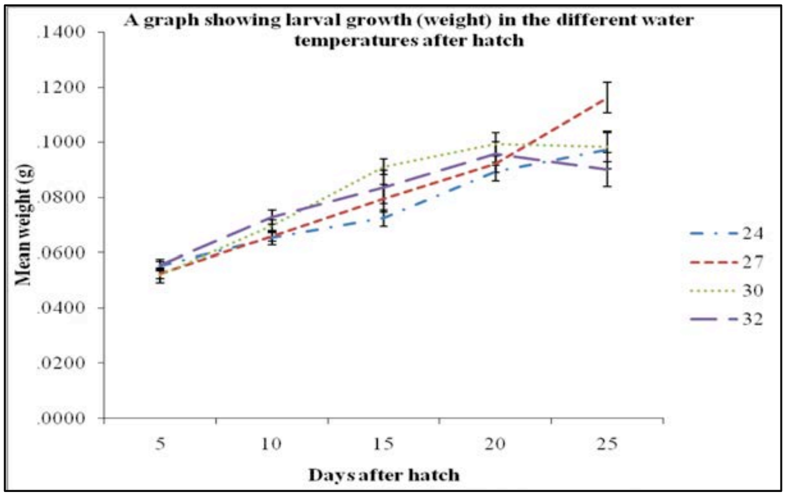

Figure 12. Larval growth at different water temperatures.

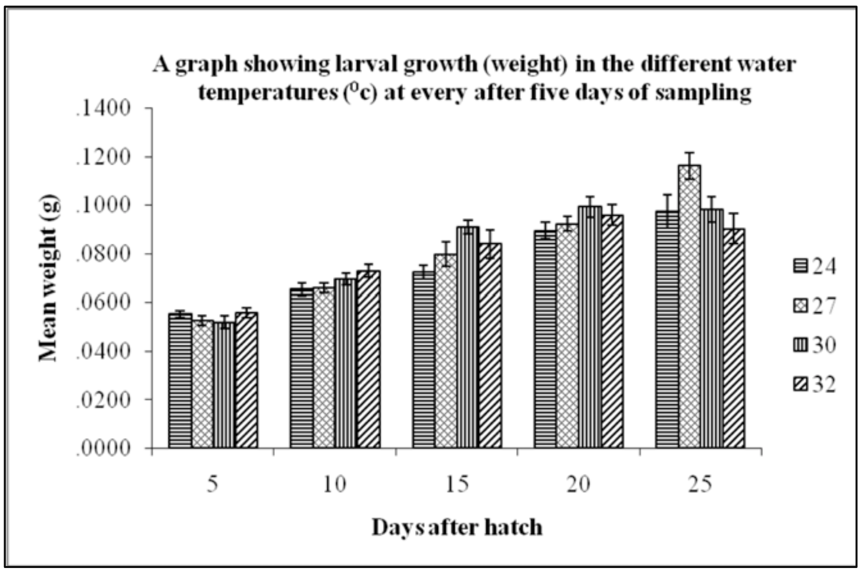

Figure 13. Lungfish larvae weight-gain at different water temperatures.

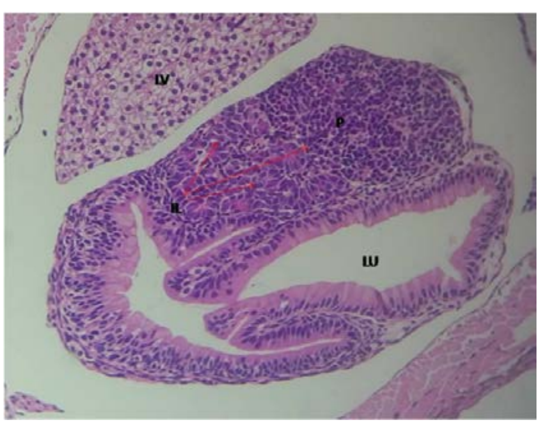

Figure 14. Histological sections of 24-DAH which shows a liver well developed with the pancreas (P) having the Islets of langerhan, the gut lumen was properly developed observed at magnification x100. Complete

exhaustion of the yolk sac, columnar epithelial cells(C), gut lumen (LU), liver (LV), IL- islets of langerhan and enterocytes (E) well observed (H&E staining).

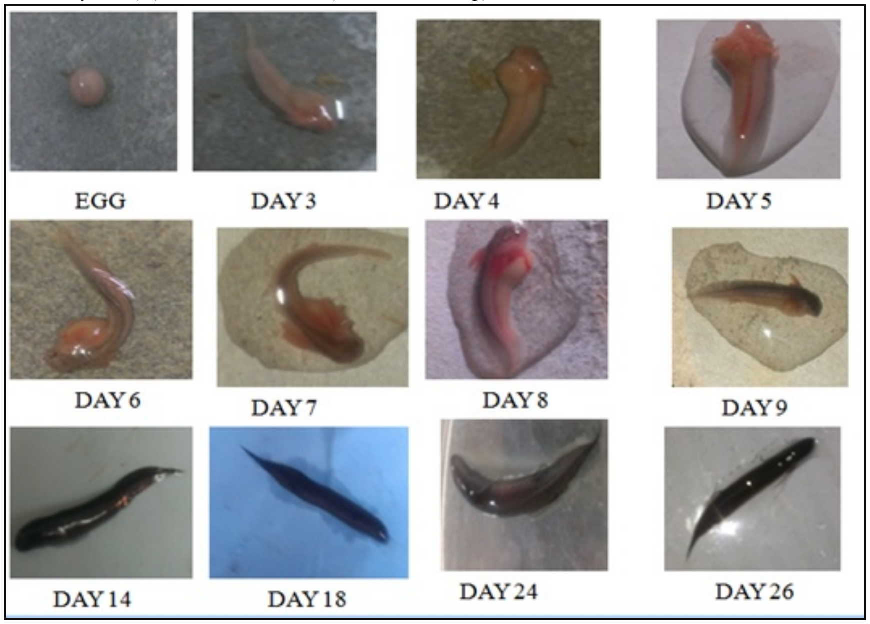

Figure 15. External development of lungfish hatchlings.

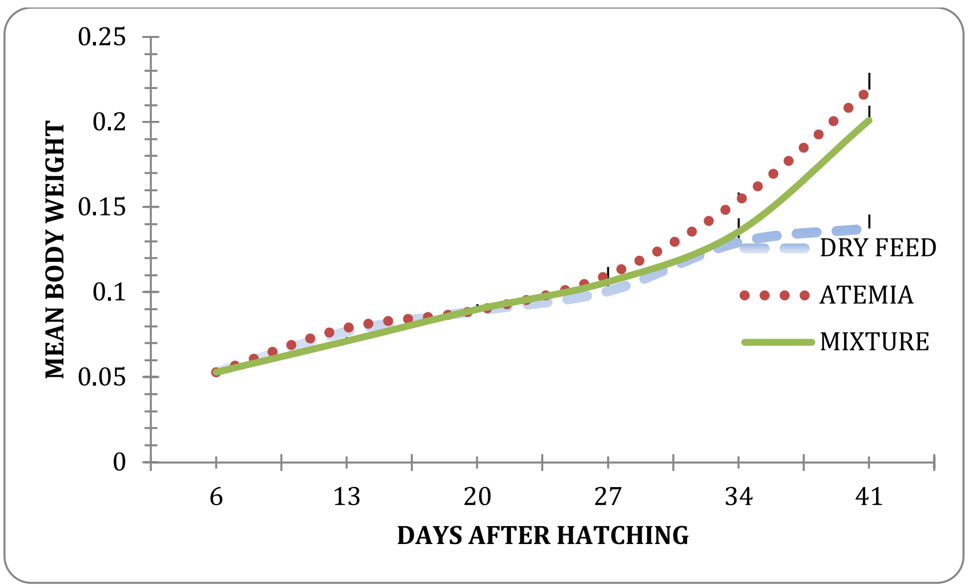

Figure 16. Growth performance of larvae weaned on three diets.

Experiment 4: Growth performance of African lungfish

Lungfish fingerlings (3.48 ± 0.83g) raised in out-door tanks and fed at *ad libitum* marginally grew to 17.13 ± 2.53g in 74 days. Hence, lungfish grew at 2.21% per day, faster than when raising wild-caught lungfish fingerlings observed during Walakira et al. 2014. Survival in this experiment was 92%, slightly higher than when growing wild-caught fingerlings. However, the size achieved in this trial is not the targeted table-size fish (>250g) hence the experiment is still on-going or under observation.

DISCUSSION

The ovary biopsy technique has been used to sample small amounts of tissue from the gonads of several different species of fishes, including the striped bass (*Morone saxatilis*) and white bass (*M. chrysops*) (Sullivan et al. 1997). The gonads in these species are connected to the urogenital pore by a straight duct that extends from the posterior end of the organ to the exterior of the fish (urogenital pore), thereby allowing direct entry of the plastic cannula into the gonad. In the case of the African lungfish, the plastic cannula can be inserted several cm, however no gonadal tissues may be aspirated (Figure 1; see also Figure 2). General gross anatomy of the African lungfish abdominal cavity is presented in **Figure 3**. Upon dissection, it appears that the lungfish has an unusual paired ovary compared to that of teleosts in that the oviduct originates from the anterior portion of the organ and then transverses posterior to the urogenital pore (Figures 4 and 5). The oviduct appears to be coiled and therefore the cannula cannot be fully inserted for gonadal tissue sampling from the ovarian lamellae (Figure 2). It may be that spawning lungfish with ovulated eggs in the oviduct could be sampled in this manner, however this has not been tested as none of the stage 3 females had ovulated eggs contained within the duct at the time of capture.

The past literature (Mosille and Mainoya, 1988 and other older literature by Greenwood), although scant, has suggested that the white coiled oviduct was "ovarian fat" and although it appears to consist largely of an unknown lipid, it is a duct with a lumen as also more recently indicated by Mlewa and Green (2004). Furthermore, an anterior loop has been identified here that connects this coiled oviduct to the ovary sac containing preovulatory oocytes (Figures 5 and 6). The ovary of stage 3 female lungfish sampled contains oocytes of different developmental stages, the largest of which constitute the most advanced clutch being 3.4-3.5 mm in diameter, consistent with an asynchronous ovary described in previous literature (Mosille and Mainoya, 1988; Mlewa and Green, 2004).

Since male African lungfish induced with hormone as described in objective 2.2 produce freely flowing semen upon palpitation of the abdomen, we suggest that this feature be used during the reproductive season to sort fish by gender such as those animals that produce semen are confirmed as males and those that fail to do so, but are of reproductive size as indicated by data from objective 1.2, are considered to be females. Fish sorted in this manner may then be kept in separate tanks based on gender for use as broodstock. Furthermore, there is no evidence of hermaphroditism in this species and all individuals sampled appeared to be gonorchistic.

The unknown, white-colored lipid of the coiled oviduct appears to be consistent with either cholesterol or a wax ester and does not appear to contain substantial triacylglycerol or other types of lipids that would typically leave a "clearing field" on white cellulose paper, as a subsample of the oviduct was streaked on paper and allowed to incubate in sunlight for 1 hour, leaving no trace of a clearing field, however it did produce in a smooth waxy surface on the paper.

Furthermore, the oviduct dissolves in organic solvents used for tissue fixation. A subsample of the oviduct was collected and frozen for subsequent analysis by lipidomics if funds are available to do so. The data collected here and also in objectives 1.1, 1.2 and 1.4 provide a comprehensive description of African lungfish internal anatomy and female gonad morphology.

Temperature is a critical factor in egg /embryo hatchability and survival of fish larvae (Haylor and Mollah 1995; Bjornsson, et al. 2001; Okunsebor, 2015). Results from this study hatchability ranged from 24 to 32^{o}C were all appropriate for hatchability of *P. aethiopicus*. It is unclear why hatchability values are low but this rate has been achieved with other fish species like *Clarius gariepinus* (Mwanja et al., 2013). Low hatchability rates in cultured species are attributed to poor quality of eggs and poor hatching conditions such as water quality(thorsen et al 2003). Since the broods were collected soon after the end of dry season it is necessary to use broods during the rainy season.

Lungfish larvae should be given live and dry feed at 18 and 26 DAH, respectively since histological sections showed well developed lumen and mucosa regions. The poor growth of fry fed on dry feed may be due to the inability of the fry to digest the feed nutrients coupled with its big size which reduced the feed intake and the best performance in fry fed live feed might be as a result of increased feed intake and high nutrient digestibility coupled with the proteolytic enzymes in the artemia.

Fry fed only formulated diet were visually smaller, lying on their sides and remained dispersed at the bottom of the aquaria in comparison with those fed live diet which were more active. It was also observed that live food seemed to be more attractable to the Larvae than other diet. Larvae Fed the combination of the diets (live and formulated) had an intermediate behavior, swimming either near the bottom or the walls. The above observations were in line with reports made by Paulo *et al.,* 2004, Adebayo and Akin 2013. The best growth performance in fry fed live feed might be as a result of increased feed intake and high nutrient digestibility of artemia that is also influenced by the proteolytic enzymes in artemia. Therefore this study indicates that source of food play an important role in delivering nutrients to fish larvae, which invariably influences their behavioral tendencies and survivability.

Previous study indicated lungfish (138 ± 42.46g) grows well with survival rates of 86%, in aquaculture outdoor tanks when poly-cultured with tilapia. In this trial, lungfish grew faster in out-door tanks with higher survival rates. Therefore, farmers can use this technology to raise lungfish in out-door tanks.

CONCLUSIONS

Artemia is the best feed for fry but given its cost, use of a mixture of feeds can be employed or other live feeds like moina or rotifers should be explored. Additionally various challenges are encountered in the species propagation and culture (hatchability, early fungal infections, among others) and their mitigation is paramount before accepting lungfish as a suitable candidate for aquaculture.

This project has unlocked important information on propagating, growing and conserving the biodiversity of African lungfish in Uganda. Government and regional bodies can now develop policies that will ensure protection of lungfish and communities dependent in this resource.

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Developing a Conceptual Model to Evaluate the Potential Changes in Inland Food Fish Supply Under Various Global Climate Change Scenarios

Climate change adaptation: Indigenous Species Development/Study/16IND04MS

Abigail Lynch, Emily Argo, Doug Beard

U.S. Geological Survey, 12201 Sunrise Valley Drive, Reston, VA 20192

ABSTRACT

Inland fish and fisheries are critical for food security in Burma. Burma is experiencing an increase in tropical storms and tidal surges along its coastline, the impacts of which can be severe due to the typography, shallow coastal ocean, and high population density in the region. Additionally, an increase in precipitation throughout the majority of Burma will likely cause increased flooding. In an effort to provide managers, policy makers, and development programs anticipate changes in inland wild and aquaculture fisheries, a systematic literature review was used to identify the impacts of climate change and applicable management strategies to address these impacts. Using the software Publish or Perish, search strings beginning with "("Myanmar" OR "Burma") AND ("fish" OR "fisheries" OR "aquaculture") AND "climate change" AND" were follow by any of the following: temperature, precipitation, sea level rise, monsoons, cyclones, or extreme weather events. Publication returns for each search string were copied into an Excel spreadsheet and three reviews were conducted to identify publications to read in full. Four management themes were identified (barrier vegetation, agriculture-aquaculture land use, protected areas, and coastal zone management) that could be addressed within three response levels (business as usual, low intervention, and high intervention). These response levels are based on the financial investment necessary to implement the management strategies and combinations of strategies from different levels can be employed. This systematic literature review sought to summarize management approaches and present them in a way that is useful to natural resource management agencies, development agencies, and nongovernmental organizations in Burma addressing impacts to wild and aquaculture fisheries.

INTRODUCTION

In Burma, the limited availability of reliable data on the status of fish populations constrains the ability of fisheries managers and policy makers to determine the current status of local fish stocks or even intervene when fisheries are in decline, until the system has virtually collapsed. This makes management efforts to anticipate the impacts of global change drivers (e.g., climate, land use) on fisheries resources exceeding difficult. For example, Allison et al. (2009) found that the Bangladesh and Cambodia national economies were both highly vulnerable to climate change impacts on fisheries, but they were unable to estimate similar impacts for Burma due to the lack of needed information on fishers and fisheries. Given the importance of inland fisheries to food security in Burma, anticipating the potential impacts of global change agents to these critical resources will help managers, policy makers and development programs anticipate and address potential challenges to inland fisheries and local communities, by region in Burma.

OBJECTIVE

Develop a conceptual model to examine the potential changes in inland food fish supply under various global change (e.g. climate, land-water use) scenarios.

METHODS AND MATERIALS

A systematic literature review was conducted using Publish or Perish (Harzing 2007) to initiate searches in Google Scholar. Search strings were entered under the prompt "All of the words:" and, with default settings, would return up to 1000 publications. All search strings began with "("Myanmar" OR "Burma") AND ("fish" OR "fisheries" OR "aquaculture") AND "climate change" AND" and were follow by any of the following: temperature, precipitation, sea level rise, monsoons, cyclones, or extreme weather events. Search returns included the following data number of times cited, author(s), title, year, source, publisher, article URL, cites URL, GSRant, query date, type, DOI, ISSN, citation URL, volume, issue, start page, end page, and ECC. Publication returns for each search string were copied into an Excel spreadsheet and three reviews were conducted to identify publications to read in full. In the first review, citations were removed if they met any of the following criteria: (1) lacked an article URL, (2) did not have a remotely relevant title (e.g., an article about the impact of cooking temperature on nutrient availability of a fish commonly harvested in Burma), or (3) came from a source not related to the environment or region. For the second review, the abstracts of the remaining publications were reviewed for relevance to climate change impacts or management strategies in the region with a specific focus on Burma and Bangladesh. All publications whose abstract did not indicate relevance were removed. The third review consisted of a reading of the full article and in the event the paper was not relevant that publication was also discarded from the list.

Once a final set of papers was identified, the papers were reviewed again and relevant content and the appropriate citation were identified. Once relevant content was collected, patterns in climate impacts and management responses were identified and summarized in two tables (See Table 1 and Table 2). Using the climate impacts table (Table 2), the scale and certainty of the impacts were determined for each of the following climate impacts: temperature, precipitation, sea level rise, drought, cyclones, extreme events and storm surge. For certainty, a score of zero indicated no confidence, one for some confidence, and two for high confidence. For scale, a score of zero indicated no impact, one for modest impact, and two for a high impact. The outcomes of this analysis is presented in Figure 1. The second table (Table 1) focused on the cost (low or high) of management strategies. Using the information generated by these summary tables of the systematic literature review, a management matrix was developed to illustrate the low and high cost management options for four management themes addressing climate impacts (Table 1). A poster communicating these management options is in development.

RESULTS

Scale and Certainty of Climate Impacts

While literature searches were defined using particular climate drivers, additional climate change impacts were identified that could affect fisheries with varying degrees of scale and certainty of impact (Figure 1). These included temperature, stream flow, storm surge, sea level rise, precipitation, flooding, extreme events, drought and cyclones. All climate change impacts were rated at least a one for both scale and certainty. Stream flow and storm surge had the largest scale and certainty ratings with a two for each criteria. Droughts, extreme events, and cyclones all received a two for the scale of their impacts, but a one for confidence in the certainty of those impacts. Flooding and temperature were both rated the same for scale (1.67), but the certainty of the impact for temperature impacts was high (1.44) than that for flooding (1). Precipitation and sea level rise both have the same certainty rating (1.5), but the scale of sea level rise is slightly higher (1.6) than that of precipitation (1.5). The sections below highlight the specific information on these climate impacts and how they may affect fish and fisheries.

Temperature

Increasing temperatures will pose a challenge to both wild and aquaculture fisheries; as waters warm fish abundance and distribution may change altering capture fisheries and an increase in decomposition will limit available oxygen negatively impacting aquaculture fisheries.

An increase in temperature has been demonstrated in recent years to cause an increase in the decomposition rate of aquatic vegetation, decreasing the oxygen, and reducing aquaculture production in Burma (Policarpio & Sheinkman 2015). Temperatures are expected to increase 0.5 - 2.9 degrees Celsius depending on the model used, location, and distance into the future (Rao et al. 2013; Horton et al. 2016; Shrestha & Htut 2016). Temperature is incredibly important for fish physiology, abundance, and distribution (Vivekanandan et al. 2016), and the mobility of a species will limit its ability to respond to temperature increases.

Sea Level Rise

Sea level rise is expected to be uniform across coastal Burma with the greatest impacts being felt in the Irrawaddy River Delta region. It is expected that this increase will impact productivity of natural areas such as mangroves, increase habitat for brackish water fish, and pose a risk to aquaculture infrastructure and survival of culture species that have low salinity tolerance.

Coastal areas and low-lying river deltas are at risk from sea level rise with consequences such as salt water intrusion, siltation, and land loss (Huq et al 2004). Saltwater intrusion is expected to adversely impact groundwater (Hossain & Selvanatham 2013) and the effects of sea level rise have already been felt in neighboring Bangladesh, where they have seen a reduction in available drinking water due to salinization of groundwater (Toufique & Islam 2014). It is predicted that the average rise in sea level will be similar across the Burma coast (Mandle et al. 2017), but the Irrawaddy Delta region may be particularly vulnerable to the impacts of sea level rise due to the high productivity and population density in the area (Taft & Evers 2016). A predicted 0.5m increase in sea level, for example, will cause the coastline of the Irrawaddy Delta to move 10km inland (NAPA 2012 as cited in Taft & Evers 2016). Additionally, tidal gauge data has indicated a 3.4-6mm/year increase in relative sea level (Syvitski et al. 2009 as cited in Brakenridge et al. 2017). The influx of salt water can alter the productivity of an area, such as mangroves, (Huq et al. 2004) and may also increase the habitat for brackish water fish (Najnin 2014). However, rising sea levels pose a risk to aquaculture infrastructure (Najnin 2014) and the species being cultured, which not be tolerant of increasing levels of salinity (Cochrane at al. 2009).

Precipitation and Stream Flow

An overall increase in precipitation and, therefore, stream flow is anticipated with slight variations based on geographic location and timeframe. An increase in the amount of time lowland areas are inundated with flood waters may impact inland fisheries given that many fisheries in Burma are located in floodplains.

While an overall increase in precipitation is expected, there are variations based on season, geographic location, and distance into the future (Shrestha & Htut 2016). The Bago River Basin, for example, has variable projections for precipitation and based on the anticipated changes in precipitation, the overall inflow into the Moeyingyi wetland is expected to decrease (Shrestha, Shrestha, & Datta 2017). Additionally, the central dry forest is expected to see a decrease in rainfall (Rao et al. 2013). The overall increase in precipitation will also increase the risk of flash flooding and increase the amount of time low-lying areas are inundated (NAPA 2012, as cited in Taft & Evers 2016).

The anticipated changes in precipitation are likely to cause an increase in stream flow. For example, the headwaters of the Irrawaddy River are fed by glacial melt in the Himalayas (Taft & Evers 2016). The precipitation increase that is expected during the wet season will likely cause increased flooding (Rao et al. 2013) with the Bago River Basin seeing an increase in flow during the wet season (Shrestha & Htut 2016).

Storm Surge and Cyclones
It is unclear how the frequency of cyclones will change in the future, the high storm surge and winds associated with these storms pose great risk to infrastructure for both wild and aquaculture fisheries.

The topography, shallow coastal ocean, and high population density in coastal Burma increase the scale and certainty of storm surge impacts caused by cyclones (Vivekanandan et al. 2016). In neighboring Bangladesh, fishers are particularly vulnerable to the impacts from storm surge because of the often fragile nature of fishing structure and equipment (Chowdhury et al. 2012). While the certainty about a change in frequency of cyclones is unclear, when they do happen they cause incredible damage when they do happen (Hossain & Saha 2017; Najnin 2014).

Drought and Extreme Events
It is unclear if the observed increase in extreme events is related to climate change, however, events such as drought threaten fisheries with their contribution to siltation of water bodies in neighboring Bangladesh.

While there has been a documented increase in the number of extreme events, it is not clear if this increase is directly related to climate change (Taft & Evers 2016). And while the frequency, intensity, and duration of these events is also unclear, the impact of an event will be related to the vulnerability of the area (Rao et al. 2013). For example, in Bangladesh, drought has contributed to the siltation of river beds and other water bodies, negatively impacting fisheries (Toufique & Islam 2014).

Management Options
Sea Level Rise
Aquaculture and rice are the food production sectors in Burma that will be impacted by sea level rise. Salinity intrusion, for example, may result in annual welfare losses up to US$10.59 billion on the global rice markets and Burma will likely go from exporting to importing rice, potentially causing increased food insecurity (Chen et al. 2012). Tidal inundation onto farm lands and fish ponds decrease involvement in farming, fish culture, and fish trade, reducing employment opportunities (Dasgupta et al. 2012). For freshwater aquaculture in areas vulnerable to saltwater intrusion, management response options include changing the cultured species or relocating operations (Cochrane et al 2009; Dasgupta et al. 2012). For rice, alternatives include freer trade for imports and switching to salinity tolerant rice varieties (Dasgupta et al. 2012; Chen et al. 2012).

Precipitation
As precipitation is projected to increase in Burma with most global climate models, management measures principally focus on managing and mitigating for increased flood waters. There are three major levels of management options for flood events: forecasting, adaption, and control. Flood forecasting does not require infrastructure to implement, but it does require hierarchical cooperation, integrated coastal zone management (Ahmad 2011). Flood adaptation measures include increasing natural vegetation to reduce inland flood risks (Mandle et al. 2017); converting farmland to fish ponds in flood prone areas and selectively cultivating quick-growing agricultural crops for production immediately after floods (Huq and Ahmed 1996); implementing integrated cropping systems (e.g., rice-fish farming; SeinnSeinn et al. 2015); and flood proofing and retrofitting buildings, roads, and flooding shelters (Kulatunga 2012). Direct flood control development projects include installing tube

wells, active irrigation, and resowing crops (Dewan et al. 2003; Kulatunga et al 2012); constructing coastal embankments (Hossain and Saha 2017); and implementing large-scale dams to control major flood pulses (Brakenridge et al. 2017). These more active measures, while providing protection in one location may cause significant impacts to fish habitat, migration routes, spawning grounds, and fishery-dependent livelihoods in other locations (Hossain and Saha 2017; Dewan et al. 2003). An additional concern of note is that, beyond local impacts, increasing conflicts may arise due to potential foreign investments in water resources (Taft and Evers 2016).

Extreme Events

Burma is hit by 0.51% of the world's total tropical storms, and due to climate change effects, the incidences of tropical storms and tidal surges have increased along the coastal belt of Burma (Rahman and Rahman 2015). Coastal zone management measures range from restoring natural vegetation to reinforcing infrastructure and implementing warning systems and community shelters (Hasan 2015). Traditional and indigenous practices, such as reinforcing building structures with fishing nets can be a simple measure to protect roofs from storm damage (Sarker and Azam 2012). Improving the accuracy of storm-surge predictions and cyclone warning systems can help protect the safety of fishermen and income potential in preparation for impacted fishing trips (Kausher et al. 1996; Chowdhury et al. 2012). One of the most effective policies for mitigating risks associated with extreme events is the maintenance of a buffer zone of mangrove forest as protected areas along the coast (Zöckler et al. 2013). Deep-rooted shrub type vegetation can serve as a defense of storm surge and prevent coastal erosion (Murty and Flather 1994); in particular, mangroves are highly effective of reducing coastal vulnerability to storms (Mandle et al. 2017; Aung et al. 2011). Mangrove systems, additionally, provide ecosystem services in their own right, providing timber and productive habitat for fisheries (Zöckler et al. 2013). It is important to note that sustainable forestry practices, especially in such critical regions, are essential to protect vulnerable populations living in delta regions; deforestation provides little resistance to surge flooding (Brakenridge et al. 2017). Fishers often prefer intervention to reduce immediate, short-term and long-term vulnerability to climate change, with some of these adaptation priorities community specific (Hasan 2015). However, more extensive management measures may be required for adaptation of some fishing-dependent communities, including climate-induced migration if their location is highly vulnerable to extreme events and no longer suitable for habitation (Islam 2013).

DISCUSSION

Based on the management information, four management areas emerged: barrier vegetation, agriculture to aquaculture, protected areas, and coastal zone management that could be addressed across three levels (business as usual, low intervention, and high intervention; Table 1). This information facilitated the development of a conceptual model illustrating these different management techniques (in development). The **business as usual** approach consists of maintaining the status quo. The **low intervention** approach, characterized by low financial investment required but rendering a substantial benefit. A more financially intensive management approach, or **high intervention**, takes some of these approaches a set further or implements a different management strategy.

Barrier Vegetation

If the status quo is maintained (**business as usual**), a continued loss of coastline and natural vegetation along coasts, rivers, and other water bodies. The **low intervention** approach encourages minor efforts to establish deep-rooted, shrub-type vegetation along embankments and restore coastal mangroves provide a low-cost, nature-based solution to address the loss of coastline and natural vegetation. The **high intervention** approach to address barrier vegetation would include intensive mangrove restoration and planting of deep-rooted, shrub-type vegetation in coastal and riparian zones.

Agriculture-Aquaculture
It is expected that there will be a loss of agriculture and aquaculture, which would cause an increase in importation of staple crops to meet demand if the **business as usual** approach is maintained. The **low intervention** approach suggests that to prevent loss of agriculture and aquaculture, the construction of embankments can help protect against flooding, but it is also important to be aware that while they may prevent flooding in one location they can also cause flooding in other areas. Given the potential for saltwater intrusion, switching to salinity tolerant varieties of rice and fish will agriculture and aquaculture operations adjust to changing climate conditions. It may also be necessary to resow crops after an event such as a flood. To increase availability of freshwater, rainwater can be harvested and stored for future use. Active irrigation and controlled flooding are low cost options to decrease the impact of flooding and maintain appropriate sediment loads, while planting vegetation can also help guard against soil erosion that can adversely impact agriculture. To address concerns with agriculture and aquaculture, the **high intervention** approach incorporates the repair of drainage systems and flood embankments and in flood-prone areas the focus would shift to integrated cropping systems (e.g., rice-fish farming) since complete conversion of agriculture to aquaculture is unlikely to be well received within Burma.

Protected Areas
The **business as usual** strategy results in a decrease in high quality habitat provided by protected areas throughout Burma. To allow high quality habitat to continue to be available, it would be necessary to maintain and enforce existing protected areas under the **low intervention** approach. To increase the availability of high quality habitat, protected area coverage could be increased within the **high intervention** approach.

Coastal Zone Management
There will also be a continued loss of life and infrastructure from storm and flood damage as well as continued poverty and poor quality of life if the current coastal zone management practices are maintained with **business as usual** strategy. Improving coastal zone management encompasses a multitude of approaches under the **low intervention** strategy, but the first step is the improvement of prediction and warning systems for flooding, storm surge, and cyclones. Additionally, providing more cyclone shelters in the event of an emergency is another low cost option that can accompany flood proofing structures by building on higher ground, reinforcing structures to withstand cyclones (e.g. using fishing nets to protect roofs from high winds), and developing flood control projects such as low lift pumps and tube wells. Finally, a **high intervention** approach to coastal zone management would include improving the construction of coastal embankments, relocation of residents out of vulnerable areas, and improving education systems, reducing poverty, enforcing regulations, and providing alternative livelihoods.

CONCLUSIONS AND LESSONS LEARNED

This work summarizes management approaches and seeks to present them in a way that is applicable to colleagues in Burma addressing the impacts of climate change on wild and aquaculture fisheries. While we have grouped management approaches into categories of high and low, we recognize a combination of the approaches discussed may be more practical based on available resources and the needs of a given region or town. For example, one location may find that they wish to invest heavily in restoring mangroves and establishing deep-rooted, shrub-type vegetation while taking a lower investment approach with their agriculture and aquaculture practices based on the resources that are most common in the area. Alternatively, another area may choose to focus on coastal zone management and continue with business as usual in other areas.

It was also important to recognize that current regulations may make implementation of management strategies more or less desirable. In some places, it is not possible to completely convert an agricultural area to aquaculture ponds in an effort to take advantage of flooding. Additionally, community perception of management approaches must be taken into consideration because if the community believes revenue may be lost, it is unlikely to be well received.

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TABLES AND FIGURES

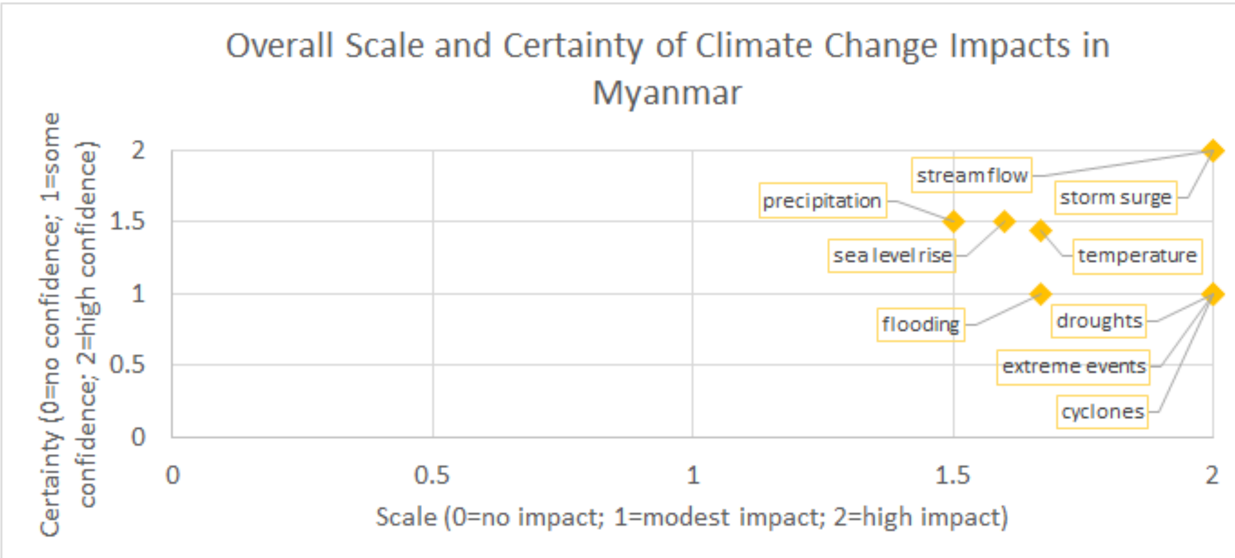

Figure 1. Numerical values for scale and certainty of climate change impacts were calculated for each source addressing a particular impact and an average of these values was used to obtain both a scale and certainty value for each impact.

Table 1. Management approaches to address climate change impacts on wild and aquaculture fisheries in Burma based on financial investment.

	BUSINESS AS USUAL	LOW INTERVENTION	HIGH INTERVENTION
BARRIER VEGETATION	Loss of coastline, natural vegetiation, etc.	Mangrove restoration (minor)	Mangrove restoration (intensive)
		Establish deep-rooted shrub type vegetation as coastal embankments in defense of storm surge can also prevent coastal erosion (minor)	Establish deep-rooted shrub type vegetation as coastal embankments in defense of storm surge can also prevent coastal erosion (intensive)
AGRICULTURE - AQUACULTURE	Loss of agriculture / aquaculture, importation of staple crops	Switch to salinity tolerant varieties of rice and fish species in coastal inundated areas	Repair and maintain flood embankment and drainage systems for agricultural and aquacultural sites
		Harvest rainwater to enhance freshwater availability	Convert farms to fish ponds in flood prone areas and focus on integrated cropping systems (e.g. rice-fish farming)
		Active irrigation	
		Controlled flooding	
		Resow crops	
PROTECTED AREAS	Loss of protected areas and high quality habitat	Enforce and maintain existing protected areas	Increase protected area coverage
COASTAL ZONE MANAGEMENT	Continued loss of life, infrastructure, etc. from storm and flooding damage; continued poverty and poor quality of life	Improved flood forcasting, storm surge predictions, and cyclone warning systems	Improve construction of coastal embankments
		Offer more cyclone shelters	Climate-induced migration (relocation of residents in vulnerable areas)
		Flood proofing (e.g., building on higher ground, retrofitting flood prone buildings, providing flooding shelters, designing roads above flood level)	Improve quality of life (education, reduce poverty, enforce regulations, providing alternative livelihoods)
		Flood control development projects (e.g., low lift pump, shallow tubewell, and improved cropping practices)	
		Reinforcing building structures with fishing nets is an indigenous practice to protect roofs from storm damage	

Table 2. Patterns in climate impacts and management responses.

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
sea level rise	adaptation and freer trade because rice is a major food staple	$$	with sea level rise, Burma goes from exporting rice to importing; as rice is a major staple, this will have a $$ effect on the economy.	Chen, C.C., McCarl, B., Chang, C.C., 2012. Climate change, sea level rise and rice: Global market implications. Clim. Change 110, 543–560. doi:10.1007/s10584-011-0074-0	Rice production is sensitive to sea level rise resulting in annual welfare losses ranging up to US$10.59 billion. The more significantly negative impacts fall in Bangladesh, Japan, Taiwan, Burma, Egypt, and Vietnam. Under the 6 m case imports rise by 292% for Bangladesh, 41% for Japan, and 3%for Taiwan. Burma, Vietnam, and Egypt go from being exporters to importers. Rice prices could be increased by 10% to 40% and depends on the effects of sea level rise and crop yield effects on rice production in each region with Bangladesh, Japan, Taiwan, Burma, and Egypt suffer from both effects. Such price increases may cause food insecurity.	Precipitation
extreme events	adaptation of fishing-dependent people	$-$$	simple adaptation measures include improvement of radio signals, weather forecasts, and warning systems; more complex adaptation measures include improving education, reducing poverty, enforcing regulations, providing alternative livelihoods	Islam, M.M., 2013. Vulnerability and Adaptation of Fishing Communities to the Impacts of Climate Variability and Change : Insights from Coastal Bangladesh Md Monirul Islam Submitted in accordance with the requirements for the degree of Doctor of Philosophy The University of. University of Leeds.	Adaptation of fishing-dependent people is impeded by both natural and anthropogenic factors: physical characteristics of climate and sea, technologically poor boats, inaccurate weather forecasts, poor radio signals, lack of access to credit, low incomes, lack of education, skills and livelihood alternatives, underestimation of cyclone occurrence, coercion of fishermen by boat owners and captains, unfavorable credit schemes, lack of enforcement of fishing regulations and maritime laws, and lack of access to fish markets.	Precipitation, Extreme Weather Events

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
extreme events	climate-induced migration	$$	migration requires considerable $$ and available livelihood strategies for positive outcomes for migrants	Islam, M.M., 2013. Vulnerability and Adaptation of Fishing Communities to the Impacts of Climate Variability and Change : Insights from Coastal Bangladesh Md Monirul Islam Submitted in accordance with the requirements for the degree of Doctor of Philosophy The University of. University of Leeds.	One direct impact of climatic shocks, such as cyclones and floods, is loss of life. Climatic shocks have killed several hundred thousand people in coastal Bangladesh, many of them fishermen or their household members, friends, or relatives (IPCC, 2007a). Other impacts include physical injuries (Badjeck et al., 2010) and health effects (Kovats et al., 2003). Cyclones and floods also damage boats, nets, fishing gear, and fish landing centres, as well as educational, health, housing and other community infrastructure (Jallow et al., 1999; Adger et al., 2005b;Westlund et al., 2007). Exposure, sensitivity, and adaptive capacity influence the vulnerability of fishery-based livelihoods in varied ways. Those who are most exposed are not necessarily the most sensitive or least able to adapt. That means the climatic stresses and shocks have unequal impacts in different fishery-dependent communities. The most important climate-related elements of exposure are floods and cyclones, while the key factor determining sensitivity of an individual household is the dependence on marine fisheries for livelihoods. Adaptive capacity is underpinned by the combination of physical, natural, and financial capital and is influenced by the diversity of livelihood strategies. The findings corroborate with the literature suggesting that climate induced migration may bring considerable positive outcomes for migrants (Black et al., 2011b) - positive livelihood outcomes were not immediate and required reduction on dependency on marine fisheries.	Precipitation, Extreme Weather Events
cyclones	Coastal zone management needs more interaction and integration with traditional ways of adaptation.	$	integrate traditional (low cost) adaptation methods into coastal zone management	Rahman, M.A., Rahman, S., 2015. Natural and traditional defense mechanisms to reduce climate risks in coastal zones of Bangladesh. Weather Clim. Extrem. 7, 84–95. doi:10.1016/j.wace.2014.12.004	Due to climate change effects, the incidences of tropical storms and tidal surges have increased in the coastal belts of Bangladesh, India, Burma and Sri Lanka; Burma is hit by 0.51% of the world's total tropical storms.	Sea Level Rise
storm surge	Coastal zone management needs more interaction and integration with traditional ways of adaptation.	$	integrate traditional (low cost) adaptation methods into coastal zone management	Rahman, M.A., Rahman, S., 2015. Natural and traditional defense mechanisms to reduce climate risks in coastal zones of Bangladesh. Weather Clim. Extrem. 7, 84–95. doi:10.1016/j.wace.2014.12.004	Due to climate change effects, the incidences of tropical storms and tidal surges have increased in the coastal belts of Bangladesh, India, Burma and Sri Lanka; Burma is hit by 0.51% of the world's total tropical storms.	Sea Level Rise
flooding	construction and improvement of coastal embankments	$	coastal embankments can protect from flooding in one location but may cause negative impacts downstream	Hossain, M.S.S., Saha, D., 2017. Major infrastructural adaptations in coastal areas of Bangladesh considering cyclone and tidal flood. Dhaka, Bangladesh.	Coastal embankments may provide protection from flooding in one location but may cause deterioration in downstream locations , creating an imbalance in sediment budget, disturbing fish habitat, and impacting livelihoods.	Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
precipitation	Controlled flooding to prevent catastrophic impacts in the future	$	utilizing the current infrastructure (dams) to implement controlled flooding to reduce sediment starvation	Brakenridge, G. R., Syvitski, J. P. M., Niebuhr, E., Overeem, I., Higgins, S. A., Kettner, A. J., & Prades, L. (2017). Design with nature: Causation and avoidance of catastrophic flooding, Myanmar. Earth-Science Reviews, 165, 81–109. https://doi.org/10.1016/j.earscirev.2016.12.009	. If large dams are now constructed upstream, it will become even more critical that some delta land areas be subject to controlled flooding in order to lesson such differential subsidence. Dam-related sediment starvation is a long term and progressive factor; like deforestation and population growth in vulnerable areas, it sets the stage for severe losses of people and economies along the coast. Addressing these more-distal causes, however, can improve the efficacy of better warnings, evacuation routes, local shelters, and increased risk awareness.	Cyclones, Monsoons, Precipitation, Sea Level Rise
all	cost of adaptation will likely be high and make fishing less profitable. Also, there is a need to promote “safety at sea” more intensively, and to incorporate disaster risk management into fisheries management plans, and fisheries management into disaster risk reduction planning	$-$$	$ measures may include introduction of a new gear type for changing conditions but most adaptation measures will be $$ because the adaptive capacity of the traditional fishing communities is quite low	Vivekanandan, E., Hermes, R., O’Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.012	The traditional fisheries will be the most vulnerable to climate change. In the east coast of India and northern Bay of Bengal, there are thousands of boats without any form of mechanization or motorization, and depend entirely on wind power for propulsion. Along the east coast of India alone there are 36,657 traditional boats (CMFRI, 2012). With no alternate income and with poor literacy, the fishermen who depend on the traditional type of fishing are faced by poverty. With restricted mobility, the competitive capacity of this fleet with other fleets is limited. The availability of fish to this fleet is also likely to decline. The traditional fisheries have demonstrated considerable resilience to climate variability in the past. For example, with the increasing abundance of small pelagics, the traditional fishers have introduced a new gear, the ring seine, for efficiently catching the oil sardine. However, any objective assessment of traditional fisheries in the region would conclude that ex- posure and sensitivity to climate change threat are high, while adaptive capacity is low (for example FAO, 2005). Among the reasons for this conclusion are:the negative impacts on the sector, e.g. through habitat and ecosystem damage such as bleaching of corals, additional stress on mangroves and sea grasses; high dependence on fishing and related activities for livelihoods; many fisher folk reside in vulnerable, low-lying coastal areas which exposes their physical assets (e.g. boats, gear, homes) and put their lives at great risk due to climate-related events such as cyclones, sea level rise and sea erosion; inadequate sea safety mechanisms to protect from extreme events, which are projected to become more frequent and/or intense in the future; and factors such as lack of consistent access to capital on reasonable terms, weak fisher folk organizations and consequently low bargaining power with governments and other sectors.	Monsoons

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
cyclones	cyclone warning system	$	Institutionalizing a better cyclone warning system will be a low cost method to save lives	Chowdhury, S.R., Hossain, M.S., Shamsuddoha, M., Khan, S.M.M.H., 2012. Coastal Fishers' Livelihood in Peril: Sea Surface Temperature and Tropical Cyclones in Bangladesh. Center for Participatory Research and Development, Dhaka, Bangladesh.	While the fishermen choose not to risk lives and refrained from fishing trips, they had to accept loss of income potential. Following the issuance of a warning fishers had to come back early by abandoning the fishing trip. The unfinished trips caused a significant loss of their livelihoods, especially in the peak fishing period. The fishers claimed that they have been faced couple of warnings throughout the monsoon. By the time they could come back to safety, their investments for the purchase of food, fuel and other commodities have all been consumed, while the catch volume was much reduced than expected. Many lost their boats and nets when the weather had become stormy. Since it all started with the increase in sea surface temperature, one cannot ignore the fact that it had been essentially triggered by global warming. Perhaps, such an extreme event provides the first ever evidence that climate change and its consequences are actually affecting people's lives and livelihoods in Bangladesh, especially in the coastal areas.	Monsoons
storm surge	deep-rooted shrub type vegetation as coastal embankments in defense of storm surge can also prevent coastal erosion; dams; warning systems and community shelters;	$	low cost measures include planting shrubs against storm surge and erosion, instituting early warning systems, and creating community shelters	Murty, T.S., Flather, R.A., 1994. Impact of Storm Surges in the Bay of Bengal. J. Coast. Res. 149–161.	Though the frequency of cyclones is not highest in the Bay of Bengal, there is a disproportionately large impact of storm surges on the coast of Bangladesh because: (1) The phenomenon of recurvature of tropical cyclones in the Bay of Bengal; (2) Shallow continental shelf, especially in the eastern part of Bangladesh; (3) High tidal range; (4) Triangular shape at the head of Bay of Bengal; (5) Almost sea level orography of the Bangladesh coast, coupled with many inlets and some rivers and estuaries; (6) High density of population, especially on low lying islands.	Monsoons
all	development projects aimed at addressing food insecurity and food production shortfalls - such as fish production for domestic consumption and exports with a special emphasis on rural poverty alleviation and employment generation	$$	addressing food insecurity and food production is a major undertaking	Alam, R., Bahauddin, K.M., 2014. Mainstreaming Climate Change Adaptation into Regional Planning of Least Developed Countries: Strategy Implications for Regions in Bangladesh. Manag. Sustain. Dev. 6, 5–17. doi:10.2478/msd-2014-0001	rural poor in less developed countries are vulnerable as they depend on the productivity of climate-sensitive ecosystems for livelihoods (including fisheries)	Sea Level Rise
Sea Level Rise	Farming to aquaculture transition	$	opportunistic transition of food production systems from farming to aquaculture	Cochrane, K., De Young, C., Soto, D., Bahri, T., & Eds. (2009). Climate change implications for fisheries and aquaculture. Overview of current scientific knowledge. FAO Technical Paper, 530, 221. https://doi.org/ISSN 2070-7010	Interactions between food production systems could compound the effects of climate change on fisheries production systems but also offer opportunities. Aquaculture based livelihoods could for example be promoted in the case of salination of deltaic areas leading to loss of agricultural land.	Extreme Weather Events
flooding	Flood Control Development projects (e.g., low lift pump, shallow tubewell, and improved cropping practices)	$	flood control measures can reduce flooding at low cost (but with impact to habitat)	Dewan, A.M., Nishigaki, M., Komatsu, M., 2003. Floods in Bangladesh: a comparative hydrological investigation on two catastrophic events. J Fac Env. Sci Technol 8, 53–62.	Control measures have switched from large-scale embankments to small-scale Flood Control Development projects but these can lead to a decline and fishery and wildlife habitat	Monsoons

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
flooding	flood forecasting	$	improved flood forecasting and integrated coastal zone management	Ahmad, Q.K., 2011. Climate change and freshwater resources of Bangladesh, in: Hossian, M., Selvanatham, A. (Eds.), Climate Change and Growth in Asia. Edward Elgar Publishing, Cheltenham and Northampton, UK, pp. 62–77.	hierarchical cooperation (local, national, regional) needed for flood forecasting and integrated coastal zone management.	Precipitation
flooding	flood mitigation through rice-fish culture; convert farms to fish ponds in ares vulnerable to flooding	$	low cost measures to mitigate floods include rice-fish culture and transitioning to fish pond aquaculture	Huq, S., Ahmed, A.U., 1996. Vulnerability of Bangladesh to Climate Change and Sea Level Rise, in: Downing, T.E. (Ed.), Climate Change and World Food Security. Series I: Global Environmental Change, Vol. 37. NATO Scientific Affairs Devision, Berlin, Germany, pp. 347–379.	About 78 per cent of the agricultural value addition is created through crop production. Livestock and poultry account for 7.6 per cent of agricultural incomes; fisheries and forestry account for 7.5 per cent and 6.6 per cent respectively. Currently, the agricultural sector (crop, livestock, forestry and fisheries) employs about 65 per cent of the labour force [in BANGLADESH]; Other means of mitigating flood effects are the cultivation of quick-growing homestead vegetables, pulses, modem short-duration mustard, maize field peas inter crop, potato under zero tillage, and rice-fish culture. These cultivation practices would intensify crop production immediately after the flood and should generate cash incomes and provide food for the affected population.	Precipitation, Sea Level Rise
flooding	Flood proofing (e.g., building on higher ground, retrofitting flood prone buildings, providing flooding shelters, designing roads above flood level) and flood forecasting	$	low cost measures to flood proof and flood forecast	Kulatunga, U., Wedawatta, G., Amaratunga, D., Parvez, A., Biswas, R., 2012. Disaster risk reduction measures in Bangladesh, in: Global Challenges in Construction Industry: World Construction Symposium, 28 – 30 June 2012. Colombo, Sri Lanka, pp. 209–220.	These are evident of ineffectiveness of embankments as flood controlling measures. On top of that, adverse impact of construction of embankments is broadened to obstruction of fish migration routes and spawning grounds, and deterioration of floodplain ecosystem	Monsoons
sea level rise	Gher is an unsustainable adaptation model - current rice breeds are not salt tolerant enough and embankment repair requires structural maintenance. The absence of a robust mechanism to prevent salinity seepage into croplands requires more research	$-$$	rice-fish culture is not always an effective transition model; more research is needed	Boiragi, K.S., 2016. Why Gher Cannot Serve as a Climate Change Adaptation Model: A Case Study on Shrimp-Rice Intercropping in Joymoni, Mongla, Bangladesh. York University.	Gher = intercropping method of cultivating paddy, shrimp and fin fish; The coastal belts are threatened primarily with Sea Level Rise (SLR), floods, cyclones, surges, erosion, and salinity intrusion; Salinity reduces soil quality, and can force farmers to use fertilizers and pesticides at extra expense. This does not bring sufficient production, but harms the natural quality of the soil further. As people do not get a good harvest from their rice paddy, they try to do gher with the idea that they could revive their losses by selling shrimp and fish. Continuous gher, instead of promising sustainable income, deteriorates the soil quality further. Ultimately farmers do not get crops, nor do they get shrimp and fin fish. Cyclones, surges, erosion, and floods exacerbate the issues	Precipitation
precipitati on	harvesting rainwater	$	inexpensive means to enhance the availability of freshwater	Ahmad, Q.K., 2011. Climate change and freshwater resources of Bangladesh, in: Hossian, M., Selvanatham, A. (Eds.), Climate Change and Growth in Asia. Edward Elgar Publishing, Cheltenham and Northampton, UK, pp. 62–77.	ponds in rural areas and rooftop facilities in urban areas can enhance the availability of freshwater.	Precipitation

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
extreme events	improve coastal home construction, offer more cyclone shelters, improve warning systems	$	simple home improvements and community activities can have large impacts on warning systems	Hasan, Z., 2015. Artisan fishers' perceptions of, and adaptation to, climate change in the southeast coast of Bangladesh. University of Adelaide.	Some of the most popular long-term adaptation techniques in response to cyclones, floods and storm surges are: ? Migration, ? Borrowing money from the local moneylenders or from friends and family members, Strengthen fishing boats and nets. ? Home improvements (for example, to raise the height of plinth), ? Strengthen nets and fishing boats, Sell off assets to get some funds to rebuild their lives,	Precipitation
flooding	improve flood forecasting and early warning; repair / maintain flood embankment and drainage systems	$-$$	from simple to long-term adaptation interventions	Hasan, Z., 2015. Artisan fishers' perceptions of, and adaptation to, climate change in the southeast coast of Bangladesh. University of Adelaide.	See Table 6.2 for adaptation preferences; Fishers' adaptation priorities indicated that fishers prefer intervention to reduce immediate, short-term and long-term vulnerability to climate change, with some of these adaptation priorities community specific	Precipitation

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
precipitation	Increase in natural vegetation/restoration/creation of protected area	$	nature-based solutions and engineering solutions to monsoon impacts	Mandle, L., Wolny, S., Bhagabati, N., Helsingen, H., Hamel, P., Bartlett, R., … Su Mon, M. (2017). Assessing ecosystem service provision under climate change to support conservation and development planning in Myanmar. PLoS ONE, 12(9), 1–23. https://doi.org/10.1371/journal.pone.0184951	Precipitation is projected to increase in most, but not all GCMs. In general, precipitation increases are projected to be largest in the monsoon season. See Supporting information (S2–S4 Data) and Horton et al. [51]for more detail on the range of projected changes in temperature and precipitation....Natural vegetation plays the greatest role in reducing inland flood risk in places with dense forest and high precipitation, where peak flow is higher....To maintain baseline levels of water quality, water availability and flood risk likely requires more than preserving existing stocks of natural capital. Nature-based solutions, such as habitat restoration, can be considered alongside traditional, engineering-based approaches [54], and further assessments could help identify the most effective places for these activities. At the same time, it is important to recognize that necessary interventions to enhance ecosystems and their resilience can be challenging to effectively complete, and do not replace the need to protect existing natural capital [55]...Our assessment therefore suggests that conservation of natural vegetation in these areas is likely to secure valuable benefits for the people of Burma today and in coming decades. We note that our analyses assume no major shifts in vegetation type or function occur over this time scale. Accounting for the effects of climate change on ES through changes in vegetation and ecological processes would be a valuable next step...By highlighting areas where loss of natural ecosystems would have outsized negative consequences for Burma's people and existing infrastructure, the national assessment can guide land use planning for agricultural expansion and resettlement plans, as well as inform the location and design of infrastructure projects...	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
storm surge	increase accuracy of storm-surge predictions	$	improve storm surge predictions	Kausher, A., Kay, R.C., Asaduzzaman, M., Paul, S., 1996. Climate Change and Sea-Level Rise: the Case of the Coast, in: Warrick, R.A., Ahmad, Q.K. (Eds.), The Implications of Climate and Sea-Level Change for Bangladesh. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 335–407.		Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
precipitati on	installing tube wells, active irrigation, resowing crops	$	install tube wells, active irrigation,	Kulatunga, U., Wedawatta, G., Amaratunga, D., Parvez, A., Biswas, R., 2012. Disaster risk reduction measures in Bangladesh, in: Global Challenges in Construction Industry: World Construction Symposium, 28 – 30 June 2012. Colombo, Sri Lanka, pp. 209–220.		Monsoons
precipitati on	integrated cropping systems (e.g., rice-fish farming) which can be environmentally secure and sustainable for household income	$	simple integrated cropping systems	SeinnSeinn, M., Ahmad, M., Thapa, G., Shrestha, R., 2015. Farmers' Adaptation to Rainfall Variability and Salinity through Agronomic Practices in Lower Ayeyarwady Delta, Myanmar. J. Earth Sci. Clim. Change 6, 258. doi:10.4172/2157-7617.1000258	Although double cropping of rice in monsoon and summer has provided higher cropping intensity and farm income, it has promoted the soil salinity and environmental unsustainability. Policy instruments are therefore suggested for an effective implementation of soil conservation and integrated farming system in lowland rainfed rice-based cropping system.	Sea Level Rise
all	International Conventions related to wetlands, biodiversity, and climate change	$$	complex international governance coordination	Gopal, B., 2013. Future of wetlands in tropical and subtropical Asia, especially in the face of climate change. Aquat. Sci. 75, 39–61. doi:10.1007/s00027-011-0247-y	The main problem with the governance and management of wetlands arises from the multiplicity of stakeholders and institutions dealing with different resources and their users. Irrigation, fisheries, agriculture, land use, water pollution, recreation are all dealt with by different institutions/organi- stations. Inadequate interagency coordination was recognized as a major barrier to a coherent management strategy for wetlands throughout the lower Mekong basin. The developed countries have started realizing the social and cultural differences and the need for community partici- pation in managing the natural resources. It is quite possible that appropriate shifts in policy will occur in response to the demands from within as well as outside the countries, and there will be greater international cooperation. The loss of ecosystem services of wetlands is likely to compel the governments and local communities to start restoration activities and change the paths of development. Successful long-term restoration and management of wetlands hinges upon our ability and capacity to manage efficiently the limited freshwater resources for meeting both human and environmental needs, coupled with our effective adaptive responses to the incremental, often synergistic, threats from climate change.	Precipitation
precipitati on	Manage foreign investment in water resources	$	increase research to understand climate change dynamics for water systems	Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	Due to the lack of scientific research in the country, of-ten uncertain or incomplete databases and rapid political and economic changes, future perspectives for human–water dynamics in Burma's river basins can only be assessed with high uncertainties. However, it should be possible to indicate the major drivers of future changes. Undoubtedly, the avail- ability and quality of freshwater is and will be the core of the country's future development, but increasing conflicts on water may arise due to growing foreign investments and various international and national interests...See Figure 3	Cyclones

Driver	**Management strategy**	**Cost ($, $$)**	**Cost rationale**	**Citation**	**Relevant content**	**Search**
storm surge	Mangrove Plantings	$	low-cost nature-based solution	Mandle, L., Wolny, S., Bhagabati, N., Helsingen, H., Hamel, P., Bartlett, R., … Su Mon, M. (2017). Assessing ecosystem service provision under climate change to support conservation and development planning in Myanmar. PLoS ONE, 12(9), 1–23. https://doi.org/10.1371/journal.pone.0184951	Mangroves are very effective at reducing coastal vulnerability to storms, especially compared to seagrass beds [50], and so the presence of man- groves (Fig 1) generally corresponded with the highest role of coastal habitat in reducing exposure	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
storm surge	Mangrove Plantings	$	low-cost nature-based solution	Aung, T. T., Than, M. M., Katsuhiro, O., & Yukira, M. (2011). Assessing the status of three mangrove species restored by the local community in the cyclone-affected area of the Ayeyarwady Delta, Myanmar. Wetlands Ecology and Management, 19(2), 195–208. https://doi.org/10.1007/s11273-011-9211-9	This study focuses on three species, Avicennia officinalis L., Heritiera fomes (Lour.) Poir. and A. marina (Forssk.) Vierh. The first two species have wide ecological amplitudes in this area (Aung et al. 2004a, b; Than et al. 2006) and seem to have a high potential for successful plantation as well as quick return to people in the form of poles, post and fuel wood, and high quality timber from H. fomes. A. marina, on the other hand, has a narrower ecological amplitude, but local people prefer it for use as poles for their construction materials...[In results section] This is consistent with the belief held bylocal people, based on their common knowledge of the region and without any special scientific investigation, that A. marina was very resistant to defoliation by Cyclone Nargis...So it is highly probable that the taller the trees in a plantation, the greater the impact by cyclones. The very low mortality of 2% for A. marina seemed to be due not only to the cyclone, but also due to effects of unfavorable site factors for this species such as drought in summer...According to our field observation, local people prefer restoring A. officinalis, rather than other species, because of its quick return benefit. If the main objective behind restoration of the plantations is utilization of the trees by local people, for example, as wood for fuel or poles other than timber, exploitable limits should be reconsidered carefully...[In conclusion section] In summary, the cyclone did not affect the survival rates of the mangrove species on these study plots to a great extent; only a slight decline was observed in A. officinalis on low ground and in A. marina on high ground. The plantations in this study are far from the seaside, and the wave effect might be negligible...During the intense cyclone of April 1991 in Bangladesh, many of the mangrove plantations were damaged, but by July 1991 most plantations showed clear signs of recovery (Siddiqi, unpublished data), except in areas of significant silt deposition. Damage to non-mangrove species raised on coastal embankments (such as Acacia nilotica) was significantly higher than that to mangrove species; the less developed root systems in non- mangrove species may have contributed to their susceptibility to 'wind-throw' (Saenger and Siddiqi 1993)...Since growth and productivity differ significantly on low versus high ground, more successful benefits for local communities can be achieved by establishing A. officinalis and A. marina on low ground and H. fomes on high ground.	Cyclones

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
extreme events	Mangrove restoration	$	low-cost nature-based solution	Zöckler, C., Delany, S., & Barber, J. (2013). Sustainable Coastal Zone Management in Myanmar. ArcCona Ecological Consultants, Cambirdge, UK.	Burma is very susceptible to extreme weather events and sea-level rise related to current and predicted future climate change. Coastal erosion and flooding are further risks which are predicted to grow...Mangroves along the Burma coast are of immediate value to local people, particularly as firewood and charcoal for cooking, timber for construction and as productive habitat for fisheries. A positive correlation between fish and shrimp catches in nearshore waters and the extent of mangrove area has been widely proven (Matosobroto & Naamin 1977; Saeskumar et al. 1992; Comach & Bagariano 1987). Artisanal fisheries along the Burma coast are largely mangrove dependent. Mangrove forest ecosystems contribute a wide range of goods and services from which local people have benefited since time immemorial. There is a wide range of direct and indirect products from mangrove, which forms the basis for mangrove dependent economic activities vital to many coastal peoples in Burma. Unsustainable exploitation has led to the depletion of many mangrove areas...Mangroves can reduce storm surge water levels by slowing the flow of water and reducing surface waves...Mangroves can therefore potentially play a role in coastal defense and disaster risk reduction, either alone or alongside other risk reduction measures such as early warning systems and engineered coastal defense structures (e.g. sea walls)(UNEP 2006). Measured rates of storm surge reduction through mangroves range from 5 to 50 centimeters water level reduction per kilometer of mangrove width. In addition, surface wind waves are expected to be reduced by more than 75% over one kilometer of mangroves...As global temperatures continue to increase, weather patterns will grow increasingly unstable and sea levels will continue to rise, causing more coastal erosion and flooding. One of the most effective policies for mitigating all these risks is the maintenance of a buffer zone of mangrove forest along the coast.	Cyclones

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
Sea Level Rise	Mangrove restoration	$	low-cost nature-based solution	Zöckler, C., Delany, S., & Barber, J. (2013). Sustainable Coastal Zone Management in Myanmar. ArcCona Ecological Consultants, Cambirdge, UK.	Burma is very susceptible to extreme weather events and sea-level rise related to current and predicted future climate change. Coastal erosion and flooding are further risks which are predicted to grow...Mangroves along the Burmacoast are of immediate value to local people, particularly as firewood and charcoal for cooking, timber for construction and as productive habitat for fisheries. A positive correlation between fish and shrimp catches in nearshore waters and the extent of mangrove area has been widely proven (Matosobroto & Naamin 1977; Saeskumar et al. 1992; Comach & Bagariano 1987). Artisanal fisheries along the Myanmar coast are largely mangrove dependent. Mangrove forest ecosystems contribute a wide range of goods and services from which local people have benefited since time immemorial. There is a wide range of direct and indirect products from mangrove, which forms the basis for mangrove dependent economic activities vital to many coastal peoples in Burma. Unsustainable exploitation has led to the depletion of many mangrove areas...Mangroves can reduce storm surge water levels by slowing the flow of water and reducing surface waves...Mangroves can therefore potentially play a role in coastal defense and disaster risk reduction, either alone or alongside other risk reduction measures such as early warning systems and engineered coastal defense structures (e.g. sea walls)(UNEP 2006). Measured rates of storm surge reduction through mangroves range from 5 to 50 centimeters water level reduction per kilometer of mangrove width. In addition, surface wind waves are expected to be reduced by more than 75% over one kilometer of mangroves...As global temperatures continue to increase, weather patterns will grow increasingly unstable and sea levels will continue to rise, causing more coastal erosion and flooding. One of the most effective policies for mitigating all these risks is the maintenance of a buffer zone of mangrove forest along the coast.	Cyclones
all	migration	$$	migration requires considerable $$ and available livelihood strategies for positive outcomes for migrants	Chowdhury, S.R., Hossain, M.S., Shamsuddoha, M., Khan, S.M.M.H., 2012. Coastal Fishers' Livelihood in Peril: Sea Surface Temperature and Tropical Cyclones in Bangladesh. Center for Participatory Research and Development, Dhaka, Bangladesh.	Migration is driven by both push and pull factors in the coastal Bangladesh. Main push factors are landscape changes caused by erosion where accretion of new land and economic solvency act as pull factors of migration. Migration is considered as a coping strategy and most migrant fishers are seasonal fishers to other coast or island places.	Monsoons
all	Modification of land use rights	$$	significant governance oversight to implement new land-use planning measures	Zöckler, C., Delany, S., & Barber, J. (2013). Sustainable Coastal Zone Management in Myanmar. ArcCona Ecological Consultants, Cambirdge, UK.	Loss of land and land rights can force local communities to turn to less sustainable harvest types and methods and can cause environmental degradation. The introduction of comprehensive land-use policies and land-use planning, consistent with sustainable livelihoods of the local communities and biodiversity conservation, will be a crucial instrument for the sustainable development of the coastal zone in Burma.	Cyclones

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
extreme events	Need to prevent deforestation or plant mangroves	$	low-cost nature-based solution	Brakenridge, G. R., Syvitski, J. P. M., Niebuhr, E., Overeem, I., Higgins, S. A., Kettner, A. J., & Prades, L. (2017). Design with nature: Causation and avoidance of catastrophic flooding, Myanmar. Earth-Science Reviews, 165, 81–109. https://doi.org/10.1016/j.earscirev.2016.12.009	Deforestation. The expansion of population in the eastern delta, where Yangon is situated, occurred together with deforestation to the west. This may have substantially increased Nargis storm damage and fatalities, via the removal of mangrove and other delta forests, and as compared to previous storms. The delta had lost much mangrove forest along the coast to shrimp farms and rice paddies in the decade prior to Nargis, and continuing a trend established for many years prior. Deforestation had long been underway: "by the late 19th century most of the dense low- land evergreen forests, swamp and mangrove forests were cleared following human settlement" (Giri et al., 2010). As the 20th century ended, the remaining forests were concentrated to the west, in the less accessible estuaries of the delta. Just before Nargis, deforestation in the Ayeyarwady delta region had accelerated: "more than 20% of the mangrove forests…lost in only 10 years; the major cause being fuel wood collection" (Leimgruber et al., 2005)(Giri et al., 2010) (Frenken, 2012).Yan- gon and its environs grew very fast in population, while the re- maining populations in the southernmost delta areas lived in a recently deforested landscape with increasingly little resistance to surge flooding. Under a "business-as-usual" scenario, some re- searchers project that unprotected Ayeyarwady delta mangrove forests could be completely deforested by 2026 (Webb, 2013). Post-Nargis, however, sustainable forestry practices could in- stead help protect the millions of inhabitants now living in the delta	Cyclones, Monsoons, Precipitation, Sea Level Rise
all	no regrets' actions	$	simple measures to reduce habitat loss, degradation, or restore or protect existing areas are "low-hanging" fruit	Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	The first set of actions involves 'no regret' actions in the absence of good biodiversity base- line and forecast data. A strategy for conserving regional biodiversity in a dynamic climate is to conserve the full spectrum of geophysical settings. If geophysical diversity helps to maintain species diversity, then conserving representative examples of geophysical settings could potentially protect biodiversity under both current and future climates (Beier and Brost 2010). Importantly, reducing or removing the effects of non-climate-related threats such as habitat loss and degradation and overexploitation will increase the ability of species and eco- systems to respond to climate change. Improving management and restoration of existing protected areas and ensuring adequate representation and replication within the protected area network will facilitate resilience.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
all	Protected areas	$-$$	can be implemented directly but will require strong safeguards to be effective	Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	Based on the above, a series of best practice principles have been actively promoted for adaptation planning that are relevant in Burma: (1) Substantially expand the current protected area system to maintain viable populations of priority species and maximize adaptive capacity; (2) Expand the current protected area system so as to capture refugia; (3) Assign priority to protecting large, intact landscapes; (4) Ensure functional connectivity is maintained beyond protected areas; (5) Develop and implement strong environmental safeguards to protect biodiversity within a context of rapidly evolving economic development in the form of large-scale infrastructure projects	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
extreme events	Protected areas	$-$$	can be implemented directly but will require strong safeguards to be effective	Zöckler, C., Delany, S., & Barber, J. (2013). Sustainable Coastal Zone Management in Myanmar. ArcCona Ecological Consultants, Cambirdge, UK.	We propose, however, that the protected area network should be expanded much more widely to include most of the Tanintharyi coast and much of the Rakhine coast. This is necessary to ensure the protection of vital ecosystem services, the last remaining marine turtle and dugong breeding sites and other important sites for wintering water birds. All these will provide essential economic incentives for the development of sustainable tourism	Cyclones
Sea Level Rise	Protected areas	$-$$	can be implemented directly but will require strong safeguards to be effective	Zöckler, C., Delany, S., & Barber, J. (2013). Sustainable Coastal Zone Management in Myanmar. ArcCona Ecological Consultants, Cambirdge, UK.	We propose, however, that the protected area network should be expanded much more widely to include most of the Tanintharyi coast and much of the Rakhine coast. This is necessary to ensure the protection of vital ecosystem services, the last remaining marine turtle and dugong breeding sites and other important sites for wintering water birds. All these will provide essential economic incentives for the development of sustainable tourism	Cyclones
cyclones	reinforcing building structures with fishing nets is an indigenous practice to protect roofs from storm damage	$	simple, low-cost measures to protect from storm damage	Sarker, T., Azam, M., 2012. Super Cyclone SIDR 2007: Climate change adaptation mechanisms for coastal communities in Bangladesh. Asian J. Environ. Disaster Manag. 4, 85–105. doi:10.3850/S1793924012001149	Devestating super cyclone on Nov. 15th, 2007 in Bangladesh - Official sources reported approximately 3500 deaths, total or partial destruction of more than 80 percent of thatched houses, damage to 70 percent of the near-harvest rice paddies, 60 to 70 percent of fisheries and huge numbers of livestock. Economic loss was estimated to be worth around Tk. 6,000 Crore which may eventually reach Tk. 16100 Crore. Damage to income generating assets included loss of fishing boats and gear.	Sea Level Rise
Sea Level Rise	Relocation of aquaculture	$	aquaculture siting based on climate projections	Cochrane, K., De Young, C., Soto, D., Bahri, T., & Eds. (2009). Climate change implications for fisheries and aquaculture. Overview of current scientific knowledge. FAO Technical Paper, 530, 221. https://doi.org/ISSN 2070-7010	...culture areas must be shifted further upstream to mitigate climatic change effects. On the other hand, climate impacts could make extra pond space available for shrimp farming, providing adequate links in the supply chains	Extreme Weather Events
sea level rise	Salinity tolerant rice varieties and fish species should be innovated and replicated in the coastal area	$	use salinity tolerant agriculture varieties to adapt to changing conditions	Dasgupta, S.K., Usami, K., Rahman, M.A., Sharifullah, A.K., 2012. Climate change and preparedness at the villege level in coastal areas of Bangladesh. Nagoya, Japan.	survey respondents indicated that tidal inundation and stagnation of saline water on farmlands and homesteads during the next 25 years will drastically decrease involvement in farming, fish culture, fishing, and fish trade and coastal communities would need to change their professions due to decreases in employment opportunities.	Precipitation

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
all	sustainable fishing practices	$-$$	win-win regardless of climate change but can be difficult to implement	Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.013	Moderately-fished stocks are likely to be more resilient to climate change impacts than heavily-fished ones. Reducing fishing mortality in the majority of fisheries, which are currently fully exploited or overexploited, is the principal means of reducing the impacts of climate change (Brander, 2007). Reduction of fishing effort (i) maximizes sustainable yields, (ii) helps adaptation of fish stocks and marine ecosystems to climate impacts, and (iii) reduces greenhouse gas emission by fishing boats (Brander, 2008). Hence, some of the most effective actions to tackle climate impacts are to deal with the familiar problems such as overfishing (Brander, 2008), and adopting implementable measures (which may differ from country to country) from the Code of Conduct for Responsible Fisheries, and an Ecosystem Approach to Fisheries Management (FAO, 2007).	Monsoons
all	Transition to more self-sustaining aquaculture strategies	$	consider more effective aquaculture practices with changing conditions	Cochrane, K., De Young, C., Soto, D., Bahri, T., & Eds. (2009). Climate change implications for fisheries and aquaculture. Overview of current scientific knowledge. FAO Technical Paper, 530, 221. https://doi.org/ISSN 2070-7010	Aquaculture systems, which are less or non-reliant on fishmeal and fish oil inputs (e.g. bivalves and macroalgae), have better scope for expansion than production systems dependent on capture fisheries commodities.	Extreme Weather Events
all	Vulnerability assessment	$-$$	conducting vulnerability assessments is one thing; implementing their recommendations is another	Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	The second category of actions involves undertaking vulnerability analyses for threatened species and ecosystem services, modeling future ecological states (accepting uncertainties) and integrating into a holistic planning framework that includes human responses to climate change impacts (Seimon et al. 2011; Cross et al. 2012). A first step is to build critically important knowledge and capacity to make climate change adaptation of conservation management effective in the absence of data. More advanced climate modeling studies are critical to under- standing climate change at relevant spatial and temporal scales in Burma. Subsequently, **scenario building exercises with scientists and stakeholders may be used to consider how outcomes may vary and what actions would be appropriate for different combinations of factors driving environmental responses to climate change**.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
storm surge				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.005	The Burma coast (Gulf of Martaban, Irrawaddy estuary) is also exposed to very damaging cyclones (e.g. cy- clone Nargis in 2008). The problem can be fathomed from the fact that during the past two and a half centuries, 20 out of 23 major cyclone disasters (with human loss of life 10,000 or more and not considering the damages) in the world have occurred over India and Bangladesh. One of the major reasons for this is the serious storm tide problem in these coasts. A tropical cyclone of specific intensity when it strikes the northern Bay of Bengal, usually produces a higher storm surge compared to that when such a cyclone strikes elsewhere in the world. This is because of the special nature of the coastline, the shallow coastal ocean topography and the characteristics of tide. Furthermore, the high density of population adds to the severity of the problem.	Monsoons
temperatu re				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.006	Sea surface temperatures (SST) have increased by 0.2–0.3 °C along the Indian coast of the Bay for the 45 year period from 1960 to 2005 (Vivekanandan et al., 2009a; Fig. 2). They have predicted increase of 2–3.5 °C by the end of the century	Monsoons
sea level rise				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.007	Sea level rise is mostly caused by thermal expansion and researchers have noted an accelerating rate of increase at 12–13 mm/decade for the northern Indian Ocean (Unnikrishnan and Shankar, 2007). The most far reaching consequences of sea level rise are threats to the coasts and coastal people through coastal erosion, inundation, and saltwater intrusion into freshwater sources and habitats, not only in Bangladesh and the Maldives, but also in Sri Lanka, Sumatra and other coastal areas of the Bay of Bengal, which may experience significantly more sea-level rise than the global mean, and this will increase the environmental stress on these coasts and islands (Han et al., 2010).	Monsoons
temperatu re				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.008	Most fish species have a fairly narrow range of optimum temperatures needed for their basic metabolism and the survival of their food supply. Being poikilotherms, even a difference of 1 °C or 0.1 unit pH in seawater may affect their physiology, abundance, and distribution. The more mobile species should be able to adjust their ranges over time, but less mobile and sedentary species may not. Depending on the species, the habitat area it occupies may expand, shrink or be relocated. As a result, the distribution of marine fish will increase in some areas, decline in others, or shift altogether.	Monsoons

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
temperatu re				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.009	The northern Indian Ocean is landlocked, and there is a natural barrier to further latitudinal shift due to the Asian continent landmass. Marine species are also inhibited by the freshwater plume of the Ganges–Brahmaputra–Megna river delta and the heavy sediment load. Warming ocean waters cause fish species to shift to deeper depths in the water column.	Monsoons
all				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.010	There is also increasing evidence of phenological changes as a result of climate change.	Monsoons
all				Vivekanandan, E., Hermes, R., O'Brien, C., 2016. Climate change effects in the Bay of Bengal Large Marine Ecosystem. Environ. Dev. 17, 46–56. doi:10.1016/j.envdev.2015.09.011	Currently, it is difficult to establish how much of catch fluctuation is due to changes in fish distribution and phenology. However, these changes may have impact on nature and value of fisheries (Perry et al., 2005). If small-sized, low-value fish species with rapid turnover of generations are able to cope with a changing climate, they may replace large-sized high-value species, which are already showing declining trends due to fishing and other non-climatic factors (Vivekanandan et al., 2005).	Monsoons
precipitati on				Shrestha, M., Shrestha, S., Datta, A., 2017. Assessment of climate change impact on water diversion from the Bago River to the Moeyingyi wetland, Myanmar. Curr. Sci. 112, 377–384. doi:10.18520/cs/v112/i02/377-384 CURRENT	In this study, the impact of climate change on water availability of the upper BRB and Moeyingyi wetland has been investigated. Two emission scenarios and three GCM outputs were used to predict the future climate data, which were used in the SWAT model to predict future discharge in the Bago River. Three future periods of up to 2050 as 2020s, 2030s and 2040s were considered for the study. The bias-corrected data of temperature show that the basin average temperature increases continuously throughout the study period, reaching up to 1.2–2.7?C, indicating an increase in evapotranspiration. It is also observed that the overall precipitation will decrease during the near future and will rise in the middle of the 21st century. The monthly analysis of precipitation also shows a decrease during the monsoon period, except in July when precipitation will increase. With increasing temperature and decreasing precipitation, a decrease in mean annual discharge will be evident at both Zangtu and Bago stations, indicating a decrease in water availability in the BRB. The projected monthly changes in discharge reveal that will decrease during May and October. These can have a serious consequences on water diversion to the Moeyingyi wetland. On the other hand, an increase in discharge during the monsoon season can worsen the regular floods in Bago city. Similarly, a large increase in discharge is predicted during April due to an increase in precipitation. Overall, the inflow at Moeyingyi wetland is expected to decrease in the future.	Precipitation

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
all				Hasan, Z., 2015. Artisan fishers' perceptions of, and adaptation to, climate change in the southeast coast of Bangladesh. University of Adelaide.	See Table 7.1 for fish catch impacts	Precipitation
sea level rise				Toufique, K.A., Islam, A., 2014. Assessing risks from climate variability and change for disaster-prone zones in Bangladesh. Int. J. Disaster Risk Reduct. 10, 236–249. doi:10.1016/j.ijdrr.2014.08.008	most fisher households (87%) in the coastal district of Bagerhat [of BANGLADESH] suffered from chronic drinking water shortages because of groundwater salinity and inadequate tube-well facilities	Monsoons, Precipitation, Sea Level Rise
flooding				Toufique, K.A., Islam, A., 2014. Assessing risks from climate variability and change for disaster-prone zones in Bangladesh. Int. J. Disaster Risk Reduct. 10, 236–249. doi:10.1016/j.ijdrr.2014.08.008	flash flood zones [in BANGLADESH] remain under water for several months, there is opportunity for fishing. Almost half of the households catch fish from the wild in this zone.	Monsoons, Precipitation, Sea Level Rise
drought				Toufique, K.A., Islam, A., 2014. Assessing risks from climate variability and change for disaster-prone zones in Bangladesh. Int. J. Disaster Risk Reduct. 10, 236–249. doi:10.1016/j.ijdrr.2014.08.008	Sandification of arable land [in BANGLADESH] results in soil degradation while fishing grounds are increasingly lost due to siltation of the river beds and other water bodies;	Monsoons, Precipitation, Sea Level Rise
cyclones				Hossain, M.S.S., Saha, D., 2017. Major infrastructural adaptations in coastal areas of Bangladesh considering cyclone and tidal flood. Dhaka, Bangladesh.	cyclone Nargis in Burma caused death of more than 138000 people (Saito and McInnes, 2014)	Sea Level Rise
flooding				Kausher, A., Kay, R.C., Asaduzzaman, M., Paul, S., 1996. Climate Change and Sea-Level Rise: the Case of the Coast, in: Warrick, R.A., Ahmad, Q.K. (Eds.), The Implications of Climate and Sea-Level Change for Bangladesh. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 335–407.	could extend the period of seasonal flooding of floodplain land which, combined with higher temperatures and increased food supplies, could extend fish breeding and growing seasons; however - human population pressure and interventions into the floodplain make these projections uncertain.	Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
sea level rise				Salmivaara, A., 2012. Myanmar: Assessing Freshwater Vulnerability in the Irrawaddy and Salween River Basins, in: Lamadrid, A., Kelman, I. (Eds.), Climate Change Modeling For Local Adaptation In The Hindu Kush-Himalayan Region (Community, Environment and Disaster Risk Management, Volume 11). Emerald Group Publishing Limited, pp. 177–206. doi:10.1108/S2040-7262(2012)0000011016	Irrawaddy delta is vulnerable in terms of low natural variability of temperature and intensive land use on wetlands that occupy most of the area; high intensive cropping and high population density - future pressures include population growth, urbanization, land-use change, and climate change influencing hydrology and sea level	Precipitation, Sea Level Rise
all				Ketelsen, T., Taylor, L., Vinh, M.K., Ma, K., Gyi, M., Charles, M., 2017. State of Knowledge: River Health in the Ayeyarwady. Vientiane, Lao PDR.	Burma government statistics indicate that inland fisheries produced 1.3 million tons of fish in 2013. Over the last decade, fisheries exports have grown steadily, at an average annual growth rate of 4.5%, and were valued at USD 653 million in 2012 (WorldFish, 2014). With climate change, fish stocks may be affected by degraded water quality, changes in fish migration, intensified competition in fishing areas and more migration of fishers.	Precipitation, Extreme Weather Events, Sea Level Rise
sea level rise				Huq, S., Reid, H., Konate, M., Rahman, A., Sokona, Y., Crick, F., 2004. Mainstreaming adaptation to climate change in Least Developed Countries (LDCs). Clim. Policy 4, 25–43. doi:10.1080/14693062.2004.9685508	Coastal zones and low lying delta areas in Asia, such as those in Bangladesh, Burma and Cambodia, are at risk from sea level rise and more frequent and severe storms due to climate change (IPCC, 1998; 2001). Deltas and estuaries will also increasingly suffer from saltwater intrusion, siltation and land loss. Sea level rise will threaten the rich biodiversity of wetlands, as it will decelerate wetland renewal. Mangroves will be affected by the rise in sea level as it will change the salinity distribution and productivity of those areas. Severe coral bleaching can be expected as a result of warmer seawater and higher incident solar radiation. The impact of global warming on fisheries will depend on how sea-level rise and changes in ocean currents affect the food chain. Increased frequency of El Niños could lead to a decline in plankton biomass and fish larvae abundance in the coastal waters of Asia, which would have a negative impact on fisheries (IPCC, 2001).	Precipitation
sea level rise				Najnin, A., 2014. Integrated Approach to Assess Vulnerability of the Coastal Region of Bangladesh due to Climate Change. Westfälische Wilhelms-Universität.	Sea-water intrusion due to sea- level rise and declining river runoff is likely to increase the habitat of brackish water fisheries but coastal inundation is likely to seriously affect the aquaculture industry and infrastructure particularly in heavily-populated mega deltas (IPCC 2007). Coastal regions are constantly subject to the action of ocean waves and storms and naturally experience erosion and inundation over various temporal and spatial scales (Westmacott 2001).	Precipitation

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
cyclones				Najnin, A., 2014. Integrated Approach to Assess Vulnerability of the Coastal Region of Bangladesh due to Climate Change. Westfälische Wilhelms-Universität.	Tropical cyclones are a major threat to the coastal areas, causing loss of human lives and livestock and severe damage to crops and fisheries. In the last 125 years, more than 42 cyclones have hit the coastal areas of Bangladesh.	Precipitation
sea level rise				Hossain, M., Selvanatham, A., 2013. Global warming induced extreme weather conditions and the threats to livelihoods in the Bay of Bengal. Int. J. Environ. 3, 1–9.	Bangladesh has doubled its fish production between 1984 and 2006; however, the achievements in the fisheries sector illustrated above are now under major threat from sea-level sea-level rise. Finan [7] painted a very alarming picture for the development of shrimp aquaculture in the coastal belt of the Bay of Bengal due to sea-level rise. In recent decades, commercial shrimp production alone has been earning more than US$350 million each year from exports. According to Finan, sea-level rise will likely result in a much larger volume of saline water moving into the canals that feed the beels (shallow water lakes), contaminating water resources and eroding gher (commercial shrimp cultivation in earthen mini-polders) embankments, which are the major sources of commercial shrimp cultivation. Another likely result of sea-level rise is saltwater intrusion through groundwater flows. This would have major adverse consequences for the groundwater irrigation system of the delta	Precipitation
cyclones				Chowdhury, S.R., Hossain, M.S., Shamsuddoha, M., Khan, S.M.M.H., 2012. Coastal Fishers' Livelihood in Peril: Sea Surface Temperature and Tropical Cyclones in Bangladesh. Center for Participatory Research and Development, Dhaka, Bangladesh.	Fishers from all the 10 locations identified windstorm, wave height and current velocity as the major climatic anomalies in the Bay of Bengal in recent years. Fishers are highly vulnerable to climate extremes because fishing implements prove to be fragile. Tropical cyclones and tidal surges may damage house, boat, fish- landing jetty, road and other physical assets that make the fishers workless. Inexperience and unavailability of other occupations can easily insecure the livelihoods of poor fishers. Sometimes they become bound for fishing even in rough weather. No alternative income generating options are reported by 99% and 97% fishers at Hatiya and Kutubdia respectively.	Monsoons
storm surge				Chowdhury, S.R., Hossain, M.S., Shamsuddoha, M., Khan, S.M.M.H., 2012. Coastal Fishers' Livelihood in Peril: Sea Surface Temperature and Tropical Cyclones in Bangladesh. Center for Participatory Research and Development, Dhaka, Bangladesh.	Fishers from all the 10 locations identified windstorm, wave height and current velocity as the major climatic anomalies in the Bay of Bengal in recent years. Fishers are highly vulnerable to climate extremes because fishing implements prove to be fragile. Tropical cyclones and tidal surges may damage house, boat, fish-landing jetty, road and other physical assets that make the fishers workless. Inexperience and unavailability of other occupations can easily insecure the livelihoods of poor fishers. Sometimes they become bound for fishing even in rough weather. No alternative income generating options are reported by 99% and 97% fishers at Hatiya and Kutubdia respectively.	Monsoons

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
all				**Horton, R., De Mel, M., Peters, D., Lesk, C., Bartlett, R., Helsingen, H., Bader, D., Capizzi, P., Martin, S. and Rosenzweig, C. 2016. Assessing Climate Risk in Myanmar. New York, NY, USA: Center for Climate Systems Research at Columbia University, WWF-US and WWF-Myanmar.; Htut AY, Shrestha S, Nitivattananon V, Kawasaki A (2014) Forecasting Climate Change Scenarios in the Bago River Basin, Myanmar. J Earth Sci Clim Change 5: 228. doi:10.4172/2157-7617.1000228**	**This report provides a great summary of the impacts of climate change in Burma with a focus on temperature, precipitation, sea level rise, and extreme weather events!**	
temperatu re				Shrestha, S., & Htut, A. Y. (2016). Land Use and Climate Change Impacts on the Hydrology of the Bago River Basin, Myanmar. Environmental Modeling & Assessment, 21(6), 819–833. https://doi.org/10.1007/s10666-016-9511-9	The seasonal results are based on three clearly distinguishable seasons in Burma: summer (JFMA), rainy (MJJA), and winter (SOND). All GCMs under both RCP scenarios indicate an increase in Tmax and Tmin for seasonal as well as annual projections. Average annual Tmax is projected to increase by 0.7 to 1.7 °C and 1.1 to 2.9 °C under RCP4.5 and 8.5 respectively. Similarly, all seasons show an increasing temperature under both scenarios in all three periods except the summer of 2020s for RCP4.5. Under the RCP8.5 scenario, all seasonal and annual changes in Tmax are projected to increase by 2.7 °C or higher in the 2080s. Similar future changes in Tmax are projected under RCP4.5 but of much smaller magnitude. Average annual Tmin is projected to rise by 1.3 and 2.5 °C under RCP4.5.and 8.5, respectively. In the case of seasonal changes, the winter of the 2050s is affected the most under RCP4.5 while it is the summer for the 2080s for RCP8.5. Future changes in Tmin are projected to be larger in magnitude under RCP8.5 than RCP4.5.	Cyclones, Precipitation, Extreme Weather Events

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
precipitati on				Shrestha, S., & Htut, A. Y. (2016). Land Use and Climate Change Impacts on the Hydrology of the Bago River Basin, Myanmar. Environmental Modeling & Assessment, 21(6), 819–833. https://doi.org/10.1007/s10666-016-9511-9	Average annual precipitation is estimatedtoincreaseby30–120 mm under RCP4.5 and by 110–125 mm under RCP8.5. As for seasonal changes, all sea- sons are likely to receive more precipitation in the future with respect to the baseline values. The winters of the 2020s and 2050s show a definite increase in precipitation by about 225 mm under RCP8.5 and 250 mm under RCP4.5. However, the increase in precipitation for the summer in the 2020s under both scenarios is relatively smaller. RCP4.5 pro- jects a greater increase in winter precipitation compared to RCP8.5. Both scenarios show similar changes in annual precipitation for the first two periods. On reaching 2080s, RCP8.5 still projects a continuous increase of precipitation, while in RCP4.5, the change is subsiding. Therefore, as per the projection, the future climate in the Bago River Basin is expected to be wetter than baseline period. The winter season is highly affected under both RCPs...[In Conclusion section] In general, an increase in precipitation was observed in the future, subject to monthly variations.	Cyclones, Precipitation, Extreme Weather Events
stream flow				Shrestha, S., & Htut, A. Y. (2016). Land Use and Climate Change Impacts on the Hydrology of the Bago River Basin, Myanmar. Environmental Modeling & Assessment, 21(6), 819–833. https://doi.org/10.1007/s10666-016-9511-9	It is observed that the stream flow within June to October is projected to increase at both stations. The peak of the stream flow is observed in September (the beginning of the winter sea- son) under both scenarios in all periods at the two stations although the baseflow peaks in August. The summer sea- son flow (January to April) is projected to remain with the same variation as the baseline period at the Bago station for all periods. However, at the Zaungtu station, the base- line stream flow is higher than the projected flow under both scenarios in the summer season. In the winter sea- son, the projected seasonal flow is higher than the base- line at the Bago station, but the Zaungtu station has a different variation (lower than the baseline) in the month of December. In 2080s, three months (October, November, and December) will witness lower stream flow at the Zaungtu station. During August, the projected stream flow is higher than the baseline period under both RCP4.5 and 8.5; however, variability is observed in September. The projected peak streamflow under RCP4.5 is higher than that of RCP8.5 in September of early and mid future at both stations...10b.In all periods, the projected stream flow is greater under the RCP8.5 scenario. Results indicate that the stream flow can increase to 350m3/s in the 2020s period under the RCP4.5 scenario and the same level in the 2080s under RCP8.5.	Cyclones, Precipitation, Extreme Weather Events

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
stream flow				Shrestha, S., & Htut, A. Y. (2016). Land Use and Climate Change Impacts on the Hydrology of the Bago River Basin, Myanmar. Environmental Modeling & Assessment, 21(6), 819–833. https://doi.org/10.1007/s10666-016-9511-10	The results show that the hydrology of the basin is more impacted by climate change compared to land use change in the near future (2020s) (Table 11). The average annual flow is projected to increase by 31–37 % and 56–58 % in Zaungtu and Bago stations, respectively, under climate change scenarios. Whereas the average annual flow is projected to increase by 10–11%and 12–13%Zaungtu and Bago stations, respectively, under land use change scenarios...This result indicates that climate change and land development alter the seasonal distributions of the stream flows rather than the change in the average annual stream flow.	Cyclones, Precipitation, Extreme Weather Events
temperature				Mandle, L., Wolny, S., Bhagabati, N., Helsingen, H., Hamel, P., Bartlett, R., … Su Mon, M. (2017). Assessing ecosystem service provision under climate change to support conservation and development planning in Myanmar. PLoS ONE, 12(9), 1–23. https://doi.org/10.1371/journal.pone.0184951	In general, slightly more warming is projected in interior regions than in southerly and coastal areas.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
Sea Level Rise				Mandle, L., Wolny, S., Bhagabati, N., Helsingen, H., Hamel, P., Bartlett, R., … Su Mon, M. (2017). Assessing ecosystem service provision under climate change to support conservation and development planning in Myanmar. PLoS ONE, 12(9), 1–23. https://doi.org/10.1371/journal.pone.0184951	Changes in average ocean height are expected to be essentially homogenous across the entire Burma coast and by season.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
precipitation				Mandle, L., Wolny, S., Bhagabati, N., Helsingen, H., Hamel, P., Bartlett, R., … Su Mon, M. (2017). Assessing ecosystem service provision under climate change to support conservation and development planning in Myanmar. PLoS ONE, 12(9), 1–23. https://doi.org/10.1371/journal.pone.0184951	Precipitation changes are likely to also alter both the amount of sediment reaching reservoirs and being retained by upstream vegetation. For the 15 reservoirs assessed in this study, sediment export to reservoirs in the absence of any land use change was estimated to decrease by an average of 3.4% (standard deviation (SD) = 1.2%) at the low end, but increase by 23.3% (SD = 3.3%) at the high end of the range of likely climate change. In addition, the amount of sediment retained upstream decreased an average of 3.4% (SD = 1.2%) under the low estimate and increased 23.3% (SD = 3.4%) under the high estimate.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
precipitation				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	The head- waters of the Ayeyarwady River are fed by glacier melt in the Himalayan Mountains and the river discharge is likely to change due to climate change impacts.	Cyclones

Driver	**Management strategy**	**Cost ($, $$)**	**Cost rationale**	**Citation**	**Relevant content**	**Search**
flooding				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	Burma's climate is directly influenced by the Indian summer monsoon (ISM; Sen Roy and Kaur, 2000; Sein et al., 2015), which is the second basic source of Burma's rivers. It is currently still not predictable whether the complex Asian monsoon circulation will strengthen, weaken or become more variable as a re- sult of global warming (Turner and Annamalai, 2012; IPCC, 2014). Already now there seems to be a trend to a delay and an earlier ending of the monsoon rains of 2 weeks in Burma respectively (Irrawaddy, 2015).	Cyclones
extreme events				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	The occurrence of extreme weather events like floods, cyclones and severe droughts has shown an increasing trend over the last 6 decades in Burma, most likely as a result of climate change (GCCA, 2012).	Cyclones
extreme events				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	Extreme weather events have become more frequent and intense during the last decades, related to their direct impacts on socio-economy, which could also be detected for Burma (GCCA, 2012). Most likely, the intensity and frequency of droughts in the dry zone, particularly during ENSO events, will increase (IPCC, 2014).	Cyclones
Sea Level Rise				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	Sea-level rise, decreasing river runoff and increasing intensity and frequency of droughts will lead to even more in- creased saltwater intrusion into river deltas...the Ayeyarwady have not been conducted yet. The low-lying Ayeyarwady delta is particularly exposed to sea-level rise and vulnerable due to its high food productivity and population density. It is assumed that a 0.5m sea-level rise would advance the shoreline along the Ayeyarwady delta by 10km in- land (NAPA, 2012).	Cyclones
temperatu re				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	In the medium term, enhanced glacier melt and snowmelt in the source areas of rivers will cause generally higher discharges and potential floods. However,	Cyclones

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
precipitation				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	Changes in river flow will likely increase the risk of flash floods and lowland regions will be regularly inundated (NAPA, 2012).	Cyclones
temperature				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	The central dry zone experienced higher maximum temperatures and less rainfall in the 1990s compared to other regions in Burma (Ministry of Forestry of the Union of Burma, 2005). This	Cyclones
precipitation				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	The central dry zone experienced higher maximum temperatures and less rainfall in the 1990s compared to other regions in Burma (Ministry of Forestry of the Union of Myanmar, 2005). This	Cyclones
precipitation				Taft, L., & Evers, M. (2016). A review of current and possible future human-water dynamics in Myanmar's river basins. Hydrology and Earth System Sciences, 20(12), 4913–4928. https://doi.org/10.5194/hess-20-4913-2016	Rao et al. (2013) concluded, based on findings from Iwamura et al. (2010), that the Ayeyarwady dry forest located in the central river basin is particularly prone to future changing rainfall and temperature conditions. The authors expect that the seasonal amount of rainfall will decrease, which will exacerbate the already water-stressed region (Rao et al., 2013).	Cyclones
extreme events				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	There is low confidence1 in any observed long-term (i.e., 40 years or more) increases in the intensity, frequency, and duration of tropical cyclone activity (IPCC 2012). However, the report indicates (with high confidence) that the severity of the impacts of climate extremes depends strongly on the level of the exposure and vulnerability to these extremes. Extreme impacts on human and ecological systems can result from individual extreme weather or climate events as well as from non-extreme events where exposure and vulnerability are high.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
extreme events				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	Burma has been ranked among the top three countries most affected by extreme weather events between 1992 and 2011 by the Global Climate Risk Index (2013) which measures the extent to which countries are affected by the impacts of weather-related events (Harmeling and Eckstein 2013).	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
extreme events				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	**Table 1** summarizes the vulnerability ratings (as high, medium, low) for the occurrence of extreme weather events (e.g., cyclones) and expectations for drought and sea-level rise in Burma based on findings reported in the Myanmar INC project (Initial National Communication Project) Report (2012). Extensive low-lying coastal areas in the south and south west appear to be highly vulnerable to impacts from floods, cyclones and associated winds and storm surges, intense rainfall and sea level rise.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
precipitation				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	Predictions for 2001–2020 based on scenarios generated for Burma show temperature increases of 0.5–0.7 ?C during the year in lower parts of Burma, record high maximum temperatures and a 4% increase in precipitation during March–November across the entire country. In particular, precipitation increases are expected in the wet season in central and north Burma. High temperatures and droughts are expected to be the norm, and are likely to be associated with more frequent forest fires in the dry zone of central Burma and the northern regions. Conversely, the increase in rainfall events in the wet season is predicted to cause flooding events which could affect livelihoods, transport, and homes.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
temperature				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	Predictions for 2001–2020 based on scenarios generated for Burma show temperature increases of 0.5–0.7 ?C during the year in lower parts of Burma, record high maximum temperatures and a 4% increase in precipitation during March–November across the entire country. In particular, precipitation increases are expected in the wet season in central and north Burma. High temperatures and droughts are expected to be the norm, and are likely to be associated with more frequent forest fires in the dry zone of central Burma and the northern regions. Conversely, the increase in rainfall events in the wet season is predicted to cause flooding events which could affect livelihoods, transport, and homes.	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise

Driver	Management strategy	Cost ($, $$)	Cost rationale	Citation	Relevant content	Search
all				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	Using the findings of Iwamura et al. (2010), we can predict the relative stability of ecoregions in Burma based on these emission scenarios **(Fig. 2**).	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
all				Rao, M., Htun, S., Platt, S. G., Tizard, R., Poole, C., Myint, T., & Watson, J. E. M. (2013). Biodiversity conservation in a changing climate: A review of threats and implications for conservation planning in myanmar. Ambio, 42(7), 789–804. https://doi.org/10.1007/s13280-013-0423-5	**Table 2**	Cyclones, Precipitation, Extreme Weather Events, Sea Level Rise
Sea Level Rise				Cochrane, K., De Young, C., Soto, D., Bahri, T., & Eds. (2009). Climate change implications for fisheries and aquaculture. Overview of current scientific knowledge. FAO Technical Paper, 530, 221. https://doi.org/ISSN 2070-7010	Catfish and rohu culture of the Mekong Delta and Irrawaddy region in Viet Nam and Burma respectively are aquaculture practices that have witnessed the highest growth ever. The regions in which these activities occur can be impacted by saline water intrusion from predicted sea level rise. The species intensively cultured at very high stocking densities and with high levels of feeding, are fresh water species with relatively low salinity tolerance. As such culture areas must be shifted further upstream to mitigate climatic change effects. On the other hand, climate impacts could make extra pond space available for shrimp farming, providing adequate links in the supply chains.	Extreme Weather Events
Sea Level Rise				Brakenridge, G. R., Syvitski, J. P. M., Niebuhr, E., Overeem, I., Higgins, S. A., Kettner, A. J., & Prades, L. (2017). Design with nature: Causation and avoidance of catastrophic flooding, Myanmar. Earth-Science Reviews, 165, 81–109. https://doi.org/10.1016/j.earscirev.2016.12.009	Tidal gauge data indicate a 3.4–6 mm/y relative sea level rise (Syvitski et al., 2009)	Cyclones, Monsoons, Precipitation, Sea Level Rise
temperatu re				Policarpio, R. R., & Sheinkman, M. (2015). State of climate information products and services for agriculture and food security in Myanmar. CCAFS Working Paper no. 140. Copenhagen, Denmark: CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS).	Freshwater and marine fisheries, and livestock are key sources of protein for Burmese households. Fisheries and livestock raising have also been affected by climate factors, such as heat stroke suffered by livestock and poultry due prolonged high temperatures; higher decomposition rate of aquatic plants resulting in reduced oxygen content in the water and reduced aquaculture production.	Cyclones, Extreme Weather Events, Sea Level Rise

TOPIC AREA

QUALITY SEEDSTOCK DEVELOPMENT

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GENETIC DIVERSITY OF STRIPED SNAKEHEAD (*CHANNA STRIATA*) IN CAMBODIA AND VIETNAM

Quality Seedstock Development/Study/16QSD01UC

So Nam[1], Chheng Phen[1], Uy Sophorn[1], Duong Thuy Yen[2], Tran Thi Thanh Hien[2] and Robert Pomeroy[3]

[1]*Inland Fisheries Research and Development Institute, Cambodia*
[2]*College of Aquaculture and Fisheries, Can Tho University, Vietnam*
[3]*Department of Agricultural and Resource Economics/CT Sea Grant, University of Connecticut-Avery Point, Groton, CT, USA*

ABSTRACT

Striped snakehead (*Channa striata*) culture has recently been allowed again in Cambodia after a ban of 12 years. In Vietnam, this species has been domesticated since 1990s. Domestication of this species in Cambodia requires genetic information to maintain good genetic resources for sustainable aquaculture. For this purpose, we collected wild samples of striped snakehead from eight locations in Cambodia including five localities around the Tonle Sap Lake (Battambang, Siem Reap, Pursat, Kampong Thom, and Kampong Chnang,) and three in the Mekong River floodplains (Kampong Cham, Kandal, and Prey Veng). We also analyzed three domesticated and wild populations in Vietnam. Sequence data of two mitochondrial markers (Cytochrome b and D-loop region) revealed that the highest level of genetic diversity was found in wild snakehead populations in Cambodia, and the lowest was in Vietnamese domesticated populations. There were no significant genetic differences among domesticated populations, but collectively domesticated populations were significantly different from wild populations. These results indicated that genetic diversity of domesticated snakehead in Vietnam decreased along with the number of domestication generations, providing useful information for snakehead farming in Cambodia. In Cambodia, suitable management of broodstock should be provided to farmers to minimize the genetic loss due to inbreeding and random genetic drift associated with the domestication process. In addition, genetic diversity of broodstock in hatcheries should be monitored periodically in order to have genetic improvement strategies in time. On the other hand, the low genetic diversity of hatchery populations in Vietnam indicated an urgent need for genetic improvement programs to prevent inbreeding depression.

INTRODUCTION

Striped snakehead (*Channa striata*) is an economically important species in both culture and capture fisheries throughout Southeast Asian countries, particularly in the Lower Mekong River basin of Cambodia and Viet Nam. Snakehead farming history in Cambodia and Viet Nam provides a typical example of species domestication for aquaculture. In Vietnam, commercial farming of striped snakehead started in the 1990s (Sinh *et al.* 2014) and rapidly developed recently due to achievements in formulated feed development (Hien *et al.* 2015, 2017a) and feeding technology, especially weaning methods for fish larvae (Hien *et al.* 2016, 2017b). In Cambodia, however, the government put a ban on snakehead farming in September 2004. Reasons for this ban were: 1) potential negative impacts on wild snakehead populations from wasteful snakehead seed collection and on other fish species diversity, particularly the freshwater small-sized fish used as feed for snakehead aquaculture, and 2) potential negative effects on poor consumer groups from decreased availability of small-sized fish due to dependence of snakehead aquaculture on small-sized fish (So 2009). The ban was lifted in June 2016 thanks to research on artificial propagation and larval and grow-out culture of striped snakehead with formulated feed (So *et al.* 2011; Nen *et al.* 2015). For sustainable aquaculture, it is critical to choose good sources with high levels of genetic diversity for domestication and breeding and appropriate broodstock management in captive conditions (Tave 1993; Dunham 2011).

Striped snakehead is a nesting-spawning and short-lived species with one-year generation time. These biological characteristics (Ellegren *et al.* 2016) and breeding techniques in captive conditions (Tave 1993; Dunham 2011) can affect species genetic diversity. The spawning season takes place from April to August, most prominently from April to June (So *et al.* 2011; Duong *et al.* 2014). In hatcheries, breeding of this species can be either semi-artificial or hormone-induced. At the main spawning season, semi-artificial methods are usually applied by farmers. One or two pairs of mature males and females are stocked in a small pond (around 10 m^2) with artificial nests using aquatic plants or artificial materials. Fish spawn after several days of daily water exchange. At a larger scale, mature fish can be induced by hormones (Marimuthu *et al.* 2007)). Striped snakehead seed production in the Viet Nam Mekong Delta is mainly supplied by local farmers at small scales (Sinh *et al.* 2014). Thus, effective breeding numbers can be predictably low, contributing to rapid decrease of genetic diversity of hatchery broodstock.

Different situations of domestication and wild sources of striped snakehead between Cambodia and Vietnam can affect differently genetic diversity of this species. In the wild, genetic diversity of populations can be threatened by over-exploitation, habitat fragmentation, introduced species, and also by interbreeding with escaped hatchery-bred individuals (Hutchings & Fraser 2008; Laikre *et al.* 2010). In aquaculture, low genetic diversity of broodstock can result in bad seed quality such as high mortality and susceptibility to diseases, or low growth rates. Long-term domestication can result in low levels of genetic diversity of hatchery-bred fish populations due to small population sizes and inappropriate broodstock management (Tave 1999; Hallerman 2008). Further genetic improvement of such populations requires genetic information of these populations and other possible sources for genetic exchange. On the other hand, in the process of domestication of potential cultured species, evaluating genetic diversity of different sources is an important step to establish good base populations (Eknath *et al.* 2007; Dunham 2011).

OBJECTIVE

Objectives include:

1. To characterize and compare genetic diversity of (1) wild (non-domesticated) snakehead populations collected from different natural water bodies in Cambodia, and (2) Cambodia wild (non-domesticated) striped snakehead and Vietnamese domesticated striped snakehead (*Channa striata*) collected from different hatcheries in the Mekong Delta, inferred from mitochondrial DNA markers.
2. To provide basic information and science-based recommendations for (1) striped snakehead domestication and selective breeding and farming in Cambodia, and (2) possible exchange of snakehead genetic resources between Cambodia and Vietnam

MATEIALS AND METHODS

Sample collection

Fish samples were collected from the wild in Cambodia and from wild and hatchery-bred populations in Vietnam (Figure 1). In Cambodia, wild (non-domesticated) snakehead samples were collected from 5 locations (including Siem Reap, SR; Battambang, BB; Kampong Thom, KT; Pursat, PS; and

Kampong Chnang, CHN) in the Tonle Sap Lake, the largest and most productive lake in South-East Asia, and three locations in the Mekong River floodplains (Kampong Cham, KCH; Kandal, KD; Prey Veng, PV). In Vietnam, fish were collected in 3 hatcheries located in the main areas of snakehead farming and reproduction (including Dong Thap DT, An Giang AG, and Hau Giang HG) and wild populations in two conservation parks (Long An LA and Ca Mau CM) and in an aquaculture area (Hau Giang HG).

Genetic analysis

DNA from fin clips from 20-30 individuals of each population was extracted using Wizard® SV Genomic DNA Purification kit (Promega, USA). DNA extracts were amplified (polymerase chain reaction, PCR) for two mitochondrial genes (mtDNA) including Cytochrome b and D-loop (or the control region) using universal primer pairs L15803/H16461 (Briolay *et al.* 1998) and D-loop-Thr-F/ D-loop-Phe-R (Cheng *et al.* 2012), respectively. The PCR thermal cycles for Cytochrome b included 1 cycle of 94°C at 2 minutes, 1 cycle of 50°C at 1 minute, 1 cycle of 71°C at 1 minute, and 38 cycles of 94°C at 30 seconds, 50°C at 30 seconds and 71°C at 30 seconds, and the last extension of 71°C at 10 minutes. D-loop was amplified at the following conditions: 1 cycle of 94°C at 4 minutes, 35 cycles of 94°C at 30 seconds, 55°C at 30 seconds, 72°C at 90 seconds, and the last step of 1 cycle of 72°C at 5 minutes (Cheng *et al.* 2012). The PCR ingredients were based on Tsigenopoulos and Berrebi (2000) for Cytochrome b and Cheng et al. (2012) for D-loop region. PCR products were visualized on 1.7% agarose gels and then sent for DNA sequencing at First BASE Laboratories Sdn Bhd (Selangor, Malaysia).

Data analysis

Sequences were first aligned by ClustalW and checked for ambiguous bases for each population using MEGA7 (Kumar *et al.* 2016) and Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). The program MEGA was employed to test the best fitting of the nucleotide substitution models based on the lowest Akaike Information Criterion (AIC) (Posada & Buckley 2004). The Tamura and Nei model with the lowest AIC was chosen for further analyses. DnaSP 5.0 (Librado & Rozas 2009) was used to estimate molecular genetic diversity indices (including the number of haplotypes, haplotype diversity, nucleotide diversity) for each population and for three groups: Cambodian, Vietnamese wild and Vietnamese domesticated populations.

For phylogenetic analyses, haplotype distribution was first obtained from Arlequin ver. 3.5 (Excoffier & Lischer 2010). Haplotype data were then constructed into a phylogenetic tree based on the Tamura and Nei model with 1000 bootstrappings (using MEGA7) and the Neighbor-Joining (NJ) tree using NETWORK software (fluxus-engineering.com) to examine the phylogenetic relationship among haplotypes.

Genetic structure of snakehead in the Lower Mekong Basin was evaluated using genetic distances and F_{st}. Genetic distances were based on the Tamura and Nei model, which was determined based on the lowest AIC when testing the best fitting of the nucleotide substitution models (Posada & Buckley 2004). Within- and between-group genetic distances were calculated using MEGA 7.0. Estimation of F_{st}-based genetic distances among populations was performed with 5000 permutations using Arlequin ver. 3.5 (Excoffier & Lischer 2010).

RESULTS

Genetic diversity of striped snakehead across populations

A total of 262 sequences of Cytochrome b and 279 sequences of D-loop with the final trimmed sequences of 585 bp and 914 bp in length, respectively, were obtained from striped snakehead wild and cultured populations in Cambodia and Vietnam. The nucleotide frequencies of Cytochrome b were 22.91% (A), 30.93% (T), 32.83% (C), and 13.33% (G), and those of D-loop were 34.32% (A); 28.94% (T); 22.08% (C), and 14.66% (G). The estimated transition/transversion bias (R) was 20.74 for Cytochrome b and 12.08 for D-loop region.

All sequences of Cytochrome b revealed 26 polymorphic sites (11 singleton variable sites and 15 parsimony informative sites) generating 28 haplotypes (Annex 1), of which 23 haplotypes were found in Cambodian wild populations, 8 haplotypes were in Vietnamese wild populations and 2 haplotypes were in Vietnamese domesticated fish (Table 2). Other molecular genetic diversity indices including haplotype diversity and nucleotide diversity of Cambodian striped snakehead populations (0.760±0.033 and 0.00239, respectively) were also highest, whereas those of Vietnamese domesticated populations were lowest (0.034±0.033 and 0.00006, respectively).

D-loop sequence data revealed similar results (Table 2). There were 128 haplotypes found in all samples with 102 haplotypes for Cambodian populations, 29 for Vietnamese wild and 5 for Vietnamese domesticated populations. Overall haplotype diversity of striped snakehead was 0.946±0.101.

At a smaller scale, genetic diversity of each population was compared with others within and between countries. In Cambodia, five populations in Tonle Sap floodplains overall had significantly higher diversity indices compared to those in the Mekong River floodplains (KCH, KD and PV) and this result was more obvious in Cytochrome b compared to D-loop sequences. In Vietnam, genetic diversity within hatchery populations was very low, especially the one in Dong Thap (DT) where there were only one haplotype of Cytochrome b and two haplotypes of D-loop region. Although Cambodian populations had higher diversity indices than wild ones in Vietnam, at least one Vietnam population (in LA) was more genetically diverse than those in KD and PV (e.g. nucleotide diversity based on Cytochrome b was 0.00152 for LA, 0.00101 for KD and 0.00045 for PV, Table 2).

Phylogeographic relationships among haplotypes
Haplotypes of Cytochrome b and D-loop were region-specific. Cambodian populations had 19 (of 28) unique haplotypes for Cytochrome b and 95/128 for D-loop, while those of Vietnamese wild populations were 4/28 and 22/128; and of Vietnamese domesticated populations were 1/28 and 2/128, respectively.

In the diagram of median-joining network based on Cytochrome b, H-3 with the highest frequency (60.31%) could be the most ancestral haplotype and was shared by three groups of populations (Figure 2). Other haplotypes presented at low frequencies (0.38-9.54%) and were not clearly structured. The most frequent D-loop haplotype was H-10 (22.22%), which is the only haplotype shared by three fish groups. Because of the large number of haplotypes (n=128) with low frequencies (0.36-2.87%), D-loop haplotypes were impractical to visualize by a network.

Genetic structure of striped snakehead in the Lower Mekong Basin
Genetic distances based on the Tamura-Nei parameter using Cytochrome b data were significant in most pairwise comparisons among wild populations, except for pairwise comparisons of domesticated populations among themselves and between these and PV in Cambodia (Annex 2). Two populations in the Mekong River floodplains in Cambodia had the smallest genetic differences with five wild and domesticated populations along the Mekong River in Vietnam (means of genetic distances range 0.0002 – 0.0010), except the CM population (0.0016) far from the River.

Values of F_{st} based on pairwise differences in Cytochrome b showed slightly different results where populations were geographically structured. Five populations in the Tonle Sap floodplains (Cambodia) were not significantly different from zero ($P>0.05$) and some of them differed ($P<0.05$)

from populations in the Lower Mekong KD and PV (Table 3). Non-significant values of F_{st} were found between KD and PV, pairwise domesticated populations, and between Vietnamese wild populations LS and HG. Cambodian KCH and Vietnam CM populations showed the highest F_{st} (ranging from 0.155-0.456 for KCH and 0.395-0.780 for CM), significantly different from all other populations (P<0.01).

Genetic distance (Annex 3) and F_{st} based on D-loop haplotypes showed some similar results. The Vietnamese population CM (but not Cambodian KCH as Cytochrome b) also had the highest values of F_{st}, ranging from 0.090-0.559 (Table 4). Low and no pairwise genetic differences were found among populations in Tonle Sap floodplains, two populations in Cambodian Mekong floodplains (KD and PV), and Vietnam domesticated fish.

However, when populations in Cambodia and Vietnam were compared, two genes revealed different inferences. Tamura-Nei genetic distances and F_{st} based on Cytochrome b indicate that Cambodian KD and PV populations were genetically closed to Vietnam populations along the Mekong River (except for CM) whereas D-loop data (Table 4 and Annex 2) showed that all Cambodian populations differed from Vietnam wild and domesticated populations (except wild HG). Domesticated populations were highly diverged in the D-loop region from most wild populations in both countries.

DISCUSSION

Genetic diversity between wild and domesticated striped snakehead populations

Results of the study revealed (i) significantly low levels of genetic diversity in domesticated striped snakehead populations in Vietnam compared to wild populations, and (ii) the overall highest genetic diversity in Cambodian wild populations. However, in smaller geographic areas, two populations in the Cambodian Mekong floodplains had lower levels of genetic diversity compared to five populations in the Tonle Sap floodplains and one Vietnam wild population.

Low genetic diversity in hatchery-bred populations has also been reported in various species such as barramundi, *Lates calcarifer* (Frost *et al.* 2006), gilthead sea bream, *Sparus aurata* (Brown *et al.* 2005), Japanese flounder, *Paralichthys olivaceus* (Sekino *et al.* 2002); and ayu, *Plecoglossus altivelis* (Iguchi *et al.* 1999). In barramundi, for example, loss of genetic diversity in cultured populations was attributed to hatchery practices including small effective population sizes of broodstock, high variation in offspring survivals and offspring contributions among breeders (Frost *et al.* 2006). These factors could also be the case for striped snakehead cultured in Vietnam. Most of the seed suppliers are small-scale farmers who own small numbers of broodstock (100-300 individuals), and a portion of fish farmers (4.3%, N= 635) can propagate seed for themselves using broodstock selected from their grow-out ponds (Sinh *et al.* 2014). Due to small breeder population sizes, genetic diversity can decrease rapidly as a result of genetic drift and inbreeding (Tave 1993; Allendorf & Luikart 2007). Negative effects of small population sizes become more severe as striped snakehead in Vietnam has been domesticated for many generations, >25 generations (domestication since 1990s with one-year generation time), evidenced by smaller haplotype diversity and nucleotide diversity than wild populations. Several other species were also reported to have low effective population sizes (N_e) in hatchery broodstock populations. For instance, N_e ranged from 14 to 18 for gilthead sea bream (Brown *et al.* 2005), or from 3 to 30 for Indian carps and thus inbreeding increased from 2% to 17% per year (Eknath & Doyle 1990). Besides, variation in offspring contribution (due to differences in offspring produced and their survival) among breeders, a common phenomenon in fish (Hedrick 2005), could lessen effective population sizes and thus lower genetic diversity of striped snakehead. In addition, differences in offspring among striped snakehead families can be caused by cannibalism behavior (Qin & Fast 1996; Abol-Munafi *et al.* 2004). Another factor that reduces N_e is unbalanced sex ratios of breeders, as reported in other aquaculture species such as gilthead sea bream, *Sparus aurata* (Brown *et al.* 2005). However, in snakehead, breeding behavior requires pairing where both parents build their nest and take care of offspring. Therefore, equal sex is also applied in artificial reproduction, and thus does not affect genetic diversity of this species.

Wild populations in the Lower Mekong River basin generally have high levels of genetic diversity compared to other fish species using similar markers. In another snakehead species *Channa marulius*, Habib et al. (2011) found that wild fish collected from three rivers in India had haplotype diversity (***Hd***) of 0.763 and nucleotide diversity (***pi***) value of 0.0128 based on sequences of 307 bp Cytochrome b mtDNA. Three populations of bighead catfish *Clarias macrocephalus* sampled in Peninsular Malaysia had lower ***pi*** (0.003) and ***Hd*** (varying from 0.657 to 0.765) (Nazia *et al.* 2010) compared to striped snakehead based on the same two genes cytochrome b and D-loop in this study (mean **pi** of two genes from 0.004 – 0.008, and **Hd** from 0.748 – 0.875). Different levels of genetic diversity among striped snakehead wild populations can be related to population sizes and different pressures of exploitation because overfishing is a main factor causing the decrease of genetic diversity in wild fish populations (Pinsky & Palumbi 2014). Among wild populations, five populations in the Tonle Sap Lake (Cambodia) showed the highest level of genetic diversity, consistent with the abundance of the species in the most productive and largest lake (Lim *et al.* 1999; Campbell *et al.* 2006).

Phylogeographic relationships and genetic structure of striped snakehead in the Lower Mekong River basin

Striped snakehead has the capacity for long-distance migration through physical connectivity (Adamson *et al.* 2010; Tan *et al.* 2012) and can also perform strong localized migration (Amilhat & Lorenzen 2005). Long migration can explain a close genetic relationship between two populations, KD and PV, in Cambodia with wild populations located along the Mekong River in Vietnam (i.e. wild HG and LA). In addition, gene flow among populations between the two countries can be caused by anthropogenic factors such as transportation along Mekong River or striped snakehead trading from Vietnam to Cambodia (Sinh *et al.* 2014). Migration of striped snakehead is also supported by the dense network of rivers and canals in the Lower Mekong basin. Although the CM population in Vietnam genetically differed from the Cambodian wild populations, data from mtDNA markers showed that the number of migrants per generation (Nm) between Cambodian striped snakehead populations with CM were less than 1 based on Cytochrome b but varied from 2-5 based on D-loop, indicating that the CM population was not isolated from the other wild populations. Further studies sampling striped snakehead from other rivers outside the Mekong River should be carried out to understand phylogeographic relationships among striped snakehead populations in the native distribution range.

Three groups (Cambodian-, Vietnam wild- and domesticated- groups) of striped snakehead had unique haplotypes for both Cytochrome b and D-loop. However, there is no relationship between geographic distances and haplotype tree or network. Most of the unique haplotypes are diverged from the common haplotype (e.g., Hap-3 in Cytochrome b), indicating they are recent mutants (Posada & Crandall 2001). Adamson et al. (2010) found that conspecific divergence of striped snakehead could have occurred in the late Miocene, about 7 million years ago.

Implications for snakehead domestication and genetic improvement

Findings on the genetic diversity of striped snakehead in the Mekong basin revealed important implications for breeding and broodstock selection of striped snakehead in Cambodia, where domestication of this species has just been started. Wild snakehead populations in the Tonle Sap can be good sources for breeding and domestication programs. A lesson from Vietnam striped snakehead farming, that genetic diversity of striped snakehead cultured populations decreases rapidly, mainly due to small effective population sizes, indicates that genetic monitoring should be regularly carried out in Cambodia during the domestication programs with the participation of small-scale fish farmers. This lesson is also useful for other fish species newly used in aquaculture. On the other hand, low genetic diversity of hatchery populations in Vietnam requires an urgent need of genetic improvement programs to prevent inbreeding depression. Replacing or supplementing local wild individuals to current hatchery broodstock can be one of the solutions (Vuorinen 1984; Garcia-Marin *et al.* 1991) in addition to increasing population sizes (Tave 1999).

Given relatively large genetic differences among Vietnamese and Cambodian populations and high levels of genetic diversity in most wild populations, genetic resource exchange of striped snakehead between the two countries is not necessary. Such action can lead to outbreeding depression (McClelland & Naish 2007; Whiteley *et al.* 2015). Moreover, results from this study have changed the intention (before genetic information was available) of using the Vietnamese domesticated strain for breeding and farming striped snakehead in Cambodia because of their superiority in growth, survival and artificial feed intake compared to Cambodian wild strains (Nen *et al.* 2015). Based on high genetic diversity, Tonle Sap populations are recommended to be used as broodstock resources for domestication in Cambodia.

CONCLUSIONS

Genetic diversity was highest in Cambodia wild populations and lowest in Vietnam domesticated populations. Domesticated populations genetically differed from wild fish in different locations. The lesson that genetic diversity of cultured snakehead in Vietnam has decreased rapidly mainly due to small-scale seed production with small effective population sizes along with a long history of domestication can be useful for snakehead farming in Cambodia. Genetic structure of the species in a large range of their distribution along the Mekong River basin was shaped by natural density of rivers/canals and anthropogenic factors.

QUANTIFIABLE ANTICIPATED BENEFITS

Cambodian and Vietnamese agencies can use this result for training fish farmers on the genetic issues in fish farming and breeding.

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TABLES AND FIGURES

Table 1. Sampling locations and population character (wild W or domesticated D) of striped snakehead populations in Cambodia and Vietnam

Locality	Abbr.	Latitude	Longtitude	Population character	Number of samples sequenced	
					Cytochrome b	D-loop
In Cambodia						
1. Siem Reap	SR	13.230908	103.835629	W	19	20
2. Battambang	BB	13.232911	103.658981	W	14	20
3. Kampong Thom	KT	12.741941	104.260975	W	19	20
4. Pursat	PS	12.568085	104.240538	W	23	20
5. Kampong Chnang	CHN	12.510229	104.449958	W	19	19
6. Kampong Cham	KCH	11.928478	105.425273	W	21	21
7. Kandal	KD	11.243789	105.023308	W	16	20
8. Prey Veng	PV	11.32384	105.287469	W	14	20
In Vietnam						
9. Long An	LA	10.774585	105.710519	W	21	20
10. Hau Giang	HG	9.726790	105.72621	W	20	20
11. Ca Mau	CM	9.273056	104.988583	W	18	20
12. An Giang	AG	10.6823	105.101584	D	20	20
13. Dong Thap	DT	10.75603	105.34919	D	17	19
14. Hau Giang	HG	9.651031	105.559292	D	21	20

Note: Locations were identified by GCS WGS84

Table 2. Summary of genetic diversity (number of haplotype **H**, haplotype diversity **Hd**, and nucleotide diversity **pi**) of snakehead populations based on Cytochrome b and D-loop sequences

Abbr.	Cytochrome b			D-loop		
	H	**Hd**	**pi**	**H**	**Hd**	**pi**
In Cambodia						
SR	6	0.784±0.059	0.00192	16	0.979±0.021	0.01234
BB	6	0.802±0.090	0.00270	15	0.968±0.00064	0.01362
KT	10	0.784±0.098	0.00270	17	0.979±0.024	0.01025
PS	10	0.866±0.049	0.00307	18	0.989±0.019	0.01305
CHN	9	0.813±0.081	0.00246	18	0.994±0.00037	0.01005
KCH	4	0.710±0.060	0.00251	8	0.829±0.00418	0.01114
KD	4	0.525±0.137	0.00101	16	0.979±0.021	0.01255
PV	2	0.264±0.136	0.00045	17	0.979±0.024	0.01136
Average	***23***	***0.760±0.033***	***0.00239***	***104***	***0.990 ± 0.002***	***0.01205***
In Vietnam						
Wild LA	2	0.095±0.084	0.00016	9	0.705±0.111	0.00689
Wild HG	8	0.647±0.120	0.00152	19	0.995±0.0003	0.01183
Wild CM	2	0.366±0.112	0.00063	8	0.816±0.071	0.00838
Average	***8***	***0.561±0.003***	***0.00152***	***29***	***0.936 ± 0.019***	***0.01058***
Domes. AG	2	0.100±0.088	0.00017	4	0.553±0.111	0.00305
Domes. DT	1	0.000±0.000	0.00000	2	0.199±0.112	0.00261
Domes. HG	1	0.000±0.000	0.00000	4	0.505±0.126	0.00375
Sub-total	***2***	***0.034±0.033***	***0.00006***	***5***	***0.431 ± 0.076***	***0.00314***
Total	**28**	**0.620±0.034**	**0.00169**	**130**	**0.946±0.0106**	**0.01104**

Note: Domes. = domestication

Table 3. Values of pairwise F_{st} based on pairwise difference in cytochrome b among striped snakehead populations in Cambodia and Vietnam (Population abbreviations are presented in Table 1)

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SR	0													
2. BB	0.0096	0												
3. KT	-0.014	-0.016	0											
4. PS	-0.013	0.046	0.033	0										
5. CHN	-0.032	-0.030	-0.030	0.007	0									
6. KCH	0.232**	0.204**	0.155**	0.222**	0.204**	0								
7. KD	0.087*	0.015	0.010	0.128**	0.010	0.254**	0							
8. PV	0.044	0.038	0.006	0.072*	0.010	0.242**	0.056	0						
9. W-LA	0.177**	0.110**	0.066**	0.187**	0.086**	0.317**	0.027	0.075	0					
10. W-HG	0.088**	0.041	0.034*	0.119**	0.037	0.219**	0.010	-0.008	0.003	0				
11. W-CM	0.487**	0.429**	0.395**	0.410**	0.420**	0.456**	0.565**	0.653**	0.731**	0.408**	0			
12. D-AG	0.172**	0.105**	0.063*	0.182**	0.087*	0.310**	0.059*	0.071	0.000	0.011	0.725**	0		
13. D-DT	0.167**	0.097**	0.054**	0.173**	0.079**	0.303**	0.059*	0.097	-0.010	0.003	0.759**	-0.008	0	
14. D-HG	0.191**	0.120**	0.070**	0.194**	0.098**	0.330**	0.077*	0.120	0.000	0.014**	0.780**	0.003	0	0

Table 4. Pairwise F_{st} based on pairwise difference in Dloop sequences among striped snakehead populations in Cambodia and Vietnam (Population abbreviations are presented in Table 1)

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1. SR	0													
2. BB	0.020	0												
3. KT	0.018	0.070*	0											
4. PS	-0.004	0.028	0.067*	0										
5. CHN	-0.014	0.042	-0.015	0.023	0									
6. KCH	0.060*	0.068**	0.017	0.084**	0.022	0								
7. KD	0.053*	0.056*	-0.003	0.086**	0.017	-0.003	0							
8. PV	0.016	0.047*	-0.005	0.025	-0.009	0.027	0.016	0						
9. W-LA	0.209**	0.226**	0.130*	0.220**	0.174**	0.112**	0.127**	0.134**	0					
10. W-HG	0.046	0.063*	0.033	0.055*	0.024	0.023	0.023	0.003	0.071*	0				
11. W-CM	0.177**	0.090**	0.212**	0.163**	0.162**	0.140**	0.132**	0.151**	0.359**	0.154**	0			
12. D-AG	0.405**	0.395**	0.321**	0.382**	0.379**	0.259**	0.311**	0.307**	0.073*	0.210**	0.559**	0		
13. D-DT	0.398**	0.389**	0.313**	0.375**	0.374**	0.252**	0.303**	0.301**	0.036	0.232**	0.554**	0.049	0	
14. D-HG	0.364**	0.363**	0.276**	0.348**	0.334**	0.228**	0.272**	0.267**	0.024	0.182**	0.524**	-0.038	-0.007	0

Annex 1. Haplotypes with positions of polimorphism of 585- bp cytochrome b

Position Haplotypes	**35**	**46**	**52**	**53**	**58**	**70**	**103**	**140**	**187**	**196**	**227**	**244**	**274**	**307**	**325**	**337**	**358**	**361**	**370**	**403**	**415**	**430**	**433**	**478**	**523**	**524**
Hap_1	G	T	T	C	C	C	C	C	G	A	C	T	C	T	G	A	C	G	C	G	G	T	G	G	T	C
Hap_2	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_3	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_4	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
Hap_5	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
Hap_6	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	.	.	.
Hap_7	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_8	A	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_9	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_10	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
Hap_11	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
Hap_12	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
Hap_13	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_14	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T
Hap_15	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
Hap_16	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
Hap_17	.	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_18	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_19	.	.	.	T	.	.	.	.	.	G	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
Hap_21	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_22	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Hap_23	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_24	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_25	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Hap_26	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
Hap_27	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
Hap_28	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Annex 2. Genetic distances (estimated using the Tamura and Nei model) based on Cytochrome b gene among Cambodian and VN (wild W, and domesticated D) populations

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1. SR		0.0009	0.0009	0.0010	0.0009	0.0013	0.0007	0.0007	0.0006	0.0007	0.0015	0.0006	0.0006	0.0006
2. BB	0.0023		0.0009	0.0011	0.0009	0.0012	0.0007	0.0007	0.0006	0.0007	0.0014	0.0006	0.0006	0.0006
3. KT	0.0023	0.0027		0.0010	0.0008	0.0012	0.0006	0.0006	0.0005	0.0006	0.0014	0.0005	0.0005	0.0005
4. PS	0.0024	0.0030	0.0030		0.0010	0.0014	0.0009	0.0009	0.0009	0.0009	0.0016	0.0009	0.0009	0.0009
5. CHN	0.0021	0.0025	0.0025	0.0028		0.0012	0.0007	0.0006	0.0005	0.0006	0.0014	0.0005	0.0005	0.0005
6. KCH	0.0029	0.0033	0.0031	0.0036	0.0031		0.0011	0.0011	0.0010	0.0011	0.0016	0.0010	0.0010	0.0010
7. KD	0.0016	0.0019	0.0019	0.0024	0.0018	0.0024		0.0004	0.0003	0.0004	0.0013	0.0003	0.0003	0.0003
8. PV	0.0013	0.0016	0.0016	0.0019	0.0015	0.0021	0.0008		0.0002	0.0004	0.0013	0.0003	0.0002	0.0002
9. W-LA	0.0013	0.0016	0.0015	0.0020	0.0014	0.0020	0.0006	0.0003		0.0003	0.0013	0.0001	0.0001	0.0001
10. W-HG	0.0019	0.0022	0.0022	0.0026	0.0021	0.0026	0.0013	0.0010	0.0008		0.0013	0.0003	0.0003	0.0003
11. W-CM	0.0025	0.0028	0.0028	0.0032	0.0027	0.0030	0.0019	0.0016	0.0014	0.0018		0.0013	0.0013	0.0013
12. D-AG	0.0013	0.0016	0.0015	0.0020	0.0014	0.0020	0.0006	0.0003	0.0002	0.0009	0.0014		0.0001	0.0001
13. D-DT	0.0012	0.0015	0.0014	0.0019	0.0014	0.0019	0.0005	0.0002	0.0001	0.0008	0.0013	0.0001		0.0000
14. D-HG	0.0012	0.0015	0.0014	0.0019	0.0014	0.0019	0.0005	0.0002	0.0001	0.0008	0.0013	0.0001	0.0000	

Annex 3. Genetic distances (estimated using the Tamura and Nei model) based on D-loop region among Cambodian and VN (wild W, and domesticated D) populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SR		0.0021	0.0019	0.0020	0.0019	0.0022	0.0021	0.0020	0.0022	0.0021	0.0022	0.0024	0.0024	0.0024
2. BB	0.0122		0.0021	0.0022	0.0021	0.0023	0.0023	0.0022	0.0024	0.0022	0.0021	0.0026	0.0026	0.0025
3. KT	0.0104	0.0130		0.0022	0.0020	0.0022	0.0021	0.0020	0.0021	0.0020	0.0022	0.0021	0.0022	0.0021
4. PS	0.0115	0.0138	0.0126		0.0021	0.0024	0.0023	0.0022	0.0024	0.0022	0.0023	0.0026	0.0026	0.0025
5. CHN	0.0099	0.0125	0.0101	0.0118		0.0022	0.0021	0.0021	0.0022	0.0020	0.0021	0.0023	0.0024	0.0023
6. KCH	0.0121	0.0141	0.0118	0.0138	0.0116		0.0023	0.0023	0.0022	0.0022	0.0023	0.0022	0.0023	0.0022
7. KD	0.0120	0.0141	0.0115	0.0141	0.0116	0.0131		0.0022	0.0022	0.0022	0.0022	0.0023	0.0023	0.0023
8. PV	0.0109	0.0133	0.0109	0.0126	0.0107	0.0124	0.0123		0.0021	0.0021	0.0022	0.0022	0.0022	0.0022
9. W-LA	0.0108	0.0134	0.0100	0.0128	0.0103	0.0111	0.0113	0.0107		0.0019	0.0025	0.0012	0.0012	0.0012
10. W-HG	0.0115	0.0138	0.0116	0.0133	0.0113	0.0125	0.0127	0.0118	0.0102		0.0022	0.0018	0.0019	0.0019
11. W-CM	0.0113	0.0123	0.0120	0.0129	0.0111	0.0126	0.0122	0.0118	0.0121	0.0121		0.0029	0.0028	0.0028
12. D-AG	0.0110	0.0140	0.0099	0.0131	0.0105	0.0110	0.0115	0.0105	0.0054	0.0095	0.0131		0.0008	0.0010
13. D-DT	0.0106	0.0136	0.0095	0.0126	0.0101	0.0107	0.0111	0.0102	0.0050	0.0096	0.0126	0.0030		0.0009
14. D-HG	0.0109	0.0138	0.0098	0.0129	0.0104	0.0109	0.0114	0.0104	0.0055	0.0096	0.0129	0.0033	0.0032	

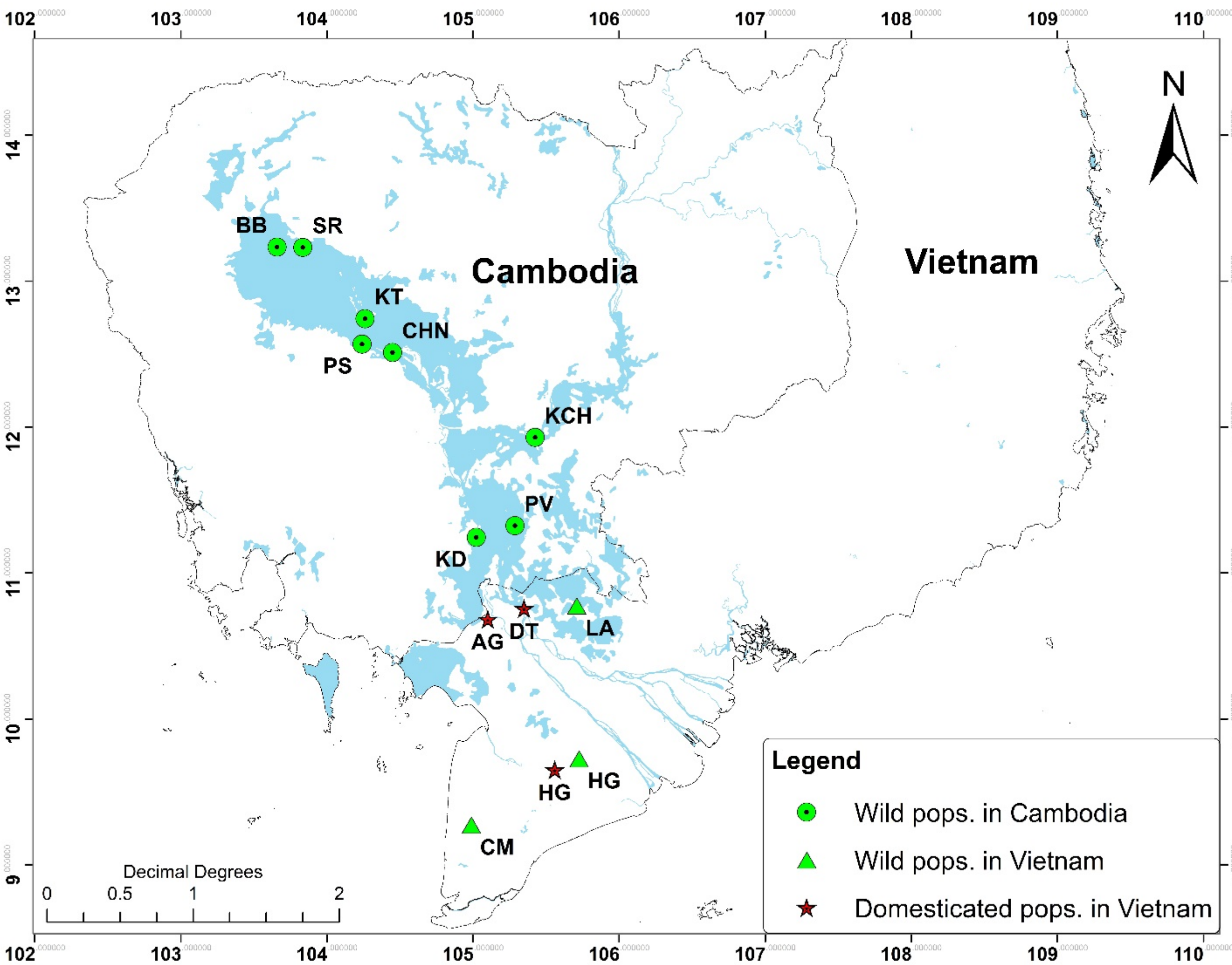

Figure 1. Sampling locations for striped snakehead in Cambodia and Vietnam.

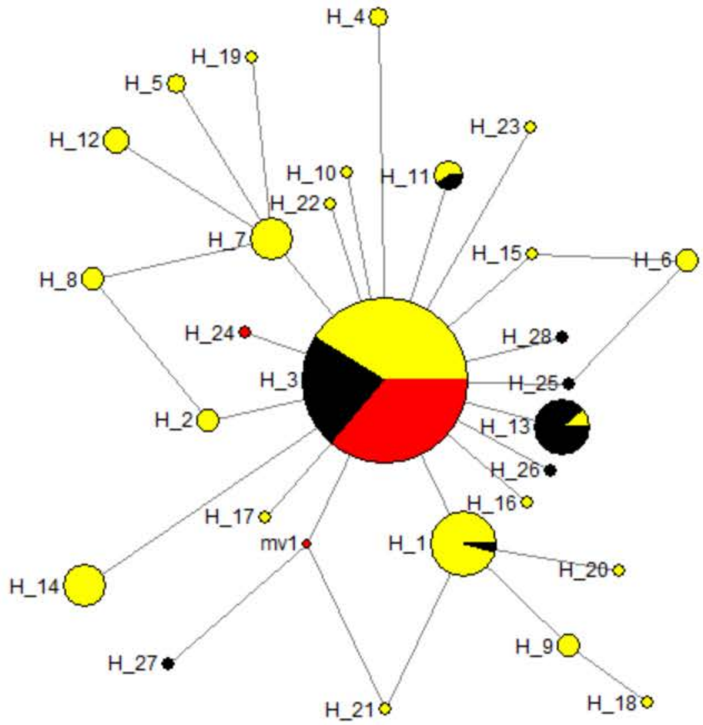

Figure 2. Median-joining network of Cytochrome b haplotypes of striped snakehead in Cambodia and Vietnam. Sizes of circles represent the haplotype frequencies. Yellow, black and red stand for Cambodian wild-, Vietnam wild- and domesticated-populations, respectively.

IMPROVING SEED PRODUCTION OF SAHAR (*TOR PUTITORA*) IN CHITWAN NEPAL

Quality Seedstock Development/Study/16QSD02UM

Jay Dev Bista[1], Rahul Ranjan[1], Narayan P. Pandit[1], Madhav K. Shrestha[1], and James S. Diana[2]

[1]*Agriculture and Forestry University, Nepal*
[2]*University of Michigan, USA*

ABSTRACT

Sahar (*Tor putitora)* is a high value indigenous riverine species of Nepal that is declining in its natural habitat and has been declared an endangered species. Limited seed production using natural propagation has restricted its expansion in culture as well as rehabilitation in natural waters. We achieved success in artificial propagation of sahar using synthetic hormone. The breeding program was conducted at the Agriculture and Forestry University, Rampur, Chitwan and the Center for Aquaculture Research and Production, Kathar, Chitwan, Nepal during February to April 2017. Sixty five male (0.5-1.8 kg) and forty five female (1.1-2.1 kg) brood fish were reared in 200 m^2 earthen ponds at 1,000 kg/ha and transferred to 25 m^2 concrete tanks one month before the breeding season. Fish were fed with 32% crude protein feed at 3% body weight per day. Maturity was observed weekly by sampling fish and testing softness of the abdomen. Female brood fish with a soft and extended abdomen were injected with synthetic hormone (Ovaprim) at 0.5 mL/kg body weight. Males did not receive any hormone injection. After 24-26 hours of injection, ova from injected females were obtained by hand stripping and fertilized with milt collected from males. The fertilized eggs were incubated in Atkins hatching trays. Sixteen females were induced to spawn, and they produced 1630.80±184.30 (mean±SE) eggs per kg body weight. Mean hatching and larval survival rates were 78.4±1.9 and 74.7±1.1%, respectively. This study demonstrated that mass seed production and larval rearing of sahar is possible in the subtropical region of Nepal using induced breeding. Induced spawning reduces the number of over-matured females by synchronizing the stripping time of injected brood fish. Fry from these spawning were reared for 90 days in hapas, and their growth was about average for that size of fish (0.7 to 1 g/d) while their survival was very high (about 95%).

INTRODUCTION

Sahar (*Tor putitora*), also known as “mahseer,” is an important fish species from the glacial water of the Himalaya. It is distributed in Trans-Himalayan countries ranging from Afghanistan to Myanmar (Skene-Dhu 1923, MacDonald 1948, Day 1958, Desai 1994, Khan et al. 1994). Sahar is a well-known, indigenous game and food fish, economically important. Sahar is widely distributed in rivers, streams, and lakes (Rai et al., 1997). While it is still taken in capture fisheries in lakes and rivers, no commercial cultivation has begun in Nepal. This species is declining from its natural habitat mainly due to over-fishing and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista et al., 2007). In recent years, success in captive breeding at some research stations and induced breeding at subtropical climates in Chitwan, Nepal have provided the opportunity to develop sahar for commercial cultivation, as well as rehabilitation in natural waters (Jha et al., 2017).

Attempts to culture and conserve sahar have been initiated in Nepal with major efforts to develop culture technology and propagate the species (Gurung et al., 2002; Joshi et al., 2002). This has led to better knowledge of spawning biology, ecology, and behavior, as well as preliminary growth performance in captive conditions. Enhanced growth in tropical and subtropical ponds, as well as the recent breeding success in hatcheries, has raised new hopes on the prospects of sahar aquaculture in Nepal (Shrestha et al., 2005; Bista et al., 2001; 2007; Rai, 2008). In addition to culture of fish to adult size for consumption, these new developments can contribute to rearing individuals that can be stocked into natural waters to replenish populations there. Due to its omnivorous and predatory feeding, sahar has also proven to be a good candidate to co-culture with mixed-sex tilapia to control tilapia recruits and provide better size at harvest and yield of tilapia (Shrestha et al., 2011). Inclusion of sahar in polyculture of mixed-sex tilapia with carps has enhanced production in these ponds.

Sahar is known to be intermittent in spawning behavior. In Nepalese context, it can mature year-round, except during January, under cultured conditions, but in natural waters, Sahar typically migrate a long distance from large rivers to streams for spawning during the monsoon (June to August), when rivers and streams are at peak flows. Programs of conservation for this fish have created increased demand for fry for restocking in rivers and lakes, as well as for aquaculture production. Insufficient availability of fish seed is a major bottleneck for commercial production and conservation.

OBJECTIVES

The overall objective of this project was to continue work to develop methods for controlled reproduction of sahar to provide seed for restocking or for aquaculture production. The specific objectives were:

1. To continue development of sahar breeding in Chitwan.
2. To improve protocols for manual detection of maturity and manual spawning of sahar.
3. To determine if gonadotropic hormone injection can be a feasible method to induce maturation of sahar.
4. To establish nursing and rearing management practices of sahar fry in Chitwan.

METHODS

The experiment for induced breeding of sahar was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur and Center for Aquaculture Research and Production (CARP), Kathar Chitwan, from September 2016 to December 2017 (Figure 1).

Figure 1. Ponds used for sahar experiments at CARP (left) and AFU (right).

Altogether 180 fish (70 females and 110 males) were reared as brood stock in a well prepared earthen pond of 200 m^2 size at CARP and in two concrete ponds each 25 m^2 in size at AFU. Females were 3 years old and approximately 1.0 kg or more body weight and males were 1+ years. They were stocked in brood ponds at the rate of 1,000 kg/ha. The male fish were always found ripe with oozing milt after pressing their belly. Brood fish were fed commercial pelleted floating feed with 32% protein at 3% of total biomass per day.

Among the 180 fish, fish were selected for spawning one month before the season. At Kathar, 30 females and 45 males were selected and kept in separate pond of 200 m^2 and 15 females and 20 males at Rampur were reared in concrete tanks of 25 m^2.

Maturity of female fish was monitored at regular intervals. They were checked biweekly before the breeding season. Male sahar can attain maturity in one year at a body size less than 50 g, but female fish first mature at the age of 3+ years. As the breeding season approached during August-November and February-April, maturity testing was increased to every third day.

For maturity tests, fish collected from ponds were held in a hammock and readiness for spawning was examined by applying gentle hand pressure near the genital opening. Ripe males ooze milt and ripe females release yellow orange eggs when stripped in this manner.

For hormonal induction of spawning, Ovaprim (Salmon Gonadotropin Releasing Hormone analogue with Domperidone) was applied at 0.5 ml/kg of female body weight as practiced in carp hatcheries in Nepal. Fully mature females with a soft belly and pinkish red vent but not ripe were induced to release eggs by hormonal injection. After 24-26 hours of injection, females were ready to spawn and eggs were stripped by pressure on the abdomen.

The females were wrapped with a soft towel for removing water from the body before stripping to avoid water drops mixing with eggs. The clean and dried females were stripped gently to collect eggs into a clean and dried bowl. Milt from males was collected in another bowl, then mixed well with eggs for dry fertilization. The fertilized eggs were washed several times and incubated in Atkins hatching trays by spreading one layer of eggs on a mesh screen in the flow-through system. Water flow was maintained at 7-9 L/minute. The incubation trays were covered with black plastic. The eggs were observed after 24 hours during incubation, and white eggs (unfertilized and dead) were counted and removed to protect healthy eggs from fungal infection. Depending on water temperature, hatching took place after 72-96 hours. Recently hatched larvae had large yolk sacs and settled around stones or near corners of the incubation tray. After attaining the free-swimming stage, larvae were transferred into a hapa of 3 m x 2 m x 1 m dimension.

Reproductive parameters, such as total number of eggs, egg number per kg body weight, fertilization rate, hatching rate, incubation period, and hatchling survival rate were also recorded to analyze breeding performance.

An experiment was conducted for 90 days (10 June – 9 September 2017) at AFU to evaluate growth performance of sahar fry. Fry were reared in fine meshed nylon hapas (2m x 1m x 1.2 m) suspended in a concrete pond. There were 4 treatments replicated thrice. The treatments were: 1) 5 fish/m^3; 2) 10 fish/m^3; 3) 15 fish/m^3; and 4) 20 fish/m^3. Stocking size of fish was 0.28-0.32 g. Fish were fed with commercial pelleted feed (32% protein content) at 5% of total biomass twice daily. The quantity of feed was adjusted fortnightly based on fish size. In situ temperature, pH, dissolved oxygen and transparency were measured weekly throughout the experimental period at 6.00-7.00 am.

RESULTS

Breeding of hormone-induced females occurred from 26 February to 4 April when water temperature ranged between 21.4°C and 28.5°C. After 24-26 hours of injection, ova from injected females were obtained by hand stripping. Sixteen females were induced to spawn and produced 2331.40±270.80 (mean±SE) eggs per female and 1630.80±184.30 eggs per kg body weight. There was a mean of 104.1±2.5 eggs per gram egg weight. Mean fertilization rate (%), incubation period (hour), hatching and larval survival rates were 96.8±1.5, 79-90, 78.4±1.9 and 74.7±1.1%, respectively (Table 1).

Table 1. Breeding performance of sahar produced by hormonal injection.

Parameter	Mean±SE
Breeding duration	2017-2-26 to 2017-4-9
Water temperature (°C)	21.4-28.5
Total no of female spawners	16
Mean weight of females (kg)	1.47±0.09
Mean weight of males (kg)	0.76±0.05
Mean total egg spawned (count)	2331.40±270.80
Mean egg number per kg body weight	1630.80±184.30
Mean egg number per gram egg weight	104.1±2.5
Mean fertilization rate (%)	96.8±1.5
Mean incubation period (hour)	79-90
Mean hatching rate (%)	78.4±1.9
Mean hatchling survival (%)	74.7±1.1

In addition to the 16 induced females, 6 naturally spawned females found during maturity testing of brood females released 115.0 g of ova. The total number of hatchlings produced from these spawnings was 8970, while 6727 fry resulted (75% survival). Regular observations from February to April revealed that only six females were overly mature and five females did not respond to hormonal injection (Table 2).

Table 2. Spawning response of sahar females that naturally matured.

Total number of spawners	6
Over mature females (no)	6
Total eggs spawned (g)	115.0
Hatchling production (no)	8970
Hatchling to fry (no)	6727

Annual water temperature of brood rearing ponds ranged from 16.2 to 31.2°C. The minimum temperature was recorded during January and maximum in July. During breeding season from September to November and February to April, the recorded temperature ranged from 19.2-26.2 and 20.4-29.5°C, respectively (Figure 2).

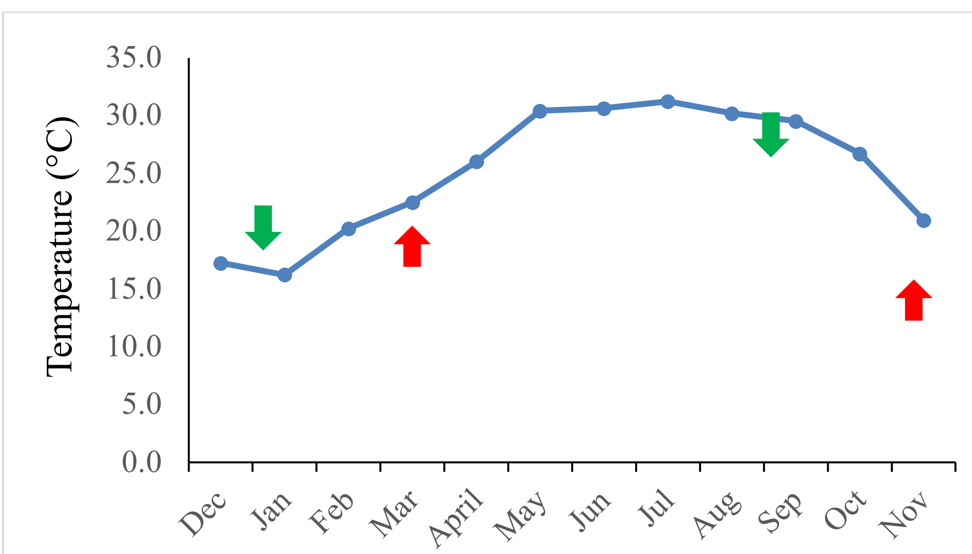

Figure 2. Annual water temperature of brood pond

Water temperature was recorded for the entire year in brood ponds. It ranged between 16.2-31.2°C. Spawning took place when temperature was between 20.2 to 26°C in February to April and 21 to 29.5°C in September to November. The first spawning occurred during a rising temperature phase while the second spawning occurred during falling temperature.

Growth and survival of sahar fry in different stocking densities indicated moderate growth rates and high survival (Table 3). Final harvest weight was significantly higher in T_1 than T_3 and T_4 ($p \leq 0.05$). There was no significant difference on survival among treatments. Growth rate of sahar fry was highest in the high density treatment, while condition factor was highest in the low density treatment (Table 4). Daily growth rate and condition factor were significantly higher in T_1 than T_3 and T_4 ($p \leq 0.05$). There was no significant difference in growth rate of fry under different treatments until 45 days growing time, but after that growth rate was significantly higher in T_1 than T_3 and T_4, where stocking density of fry was 3-4 times higher.

Table 3. Production and survival of sahar fry reared at different stocking densities

Parameter	Treatments			
	T_1 (5 fish/m^2)	T_2 (10 fish/m^2)	T_3 (15 fish/m^2)	T_4 (20 fish/m^2)
Mean stocking weight (g)	0.30 ± 0.01^a	0.29 ± 0.02^a	0.32 ± 0.02^a	0.28 ± 0.02^a
Stocking number (fish/hapa)	10.00±0.00	20.00±0.00	30.00±0.00	40.00±0.00
Average harvested weight (g)	6.13 ± 0.33^a	4.34 ± 0.98^{ab}	2.97 ± 0.55^b	2.84 ± 0.56^b
Harvest number (fish/hapa)	9.67±0.33	19.00±0.58	29.33±0.33	36.33±1.20
Survival (%)	96.7 ± 3.33^a	95.0 ± 2.89^a	97.8 ± 1.11^a	90.8 ± 3.00^a

Table 4. Growth rate of sahar fry reared at different stocking densities

Parameter	Treatments			
	T_1 (5 fish/m^2)	T_2 (10 fish/m^2)	T_3 (15 fish/m^2)	T_4 (20 fish/m^2)
Daily growth rate (g/day)	0.08 ± 0.04^a	0.05 ± 0.01^{ab}	0.04 ± 0.01^b	0.03 ± 0.01^b
Growth rate (% BW/day)	1.46 ± 0.03^a	1.27 ± 0.15^a	1.06 ± 0.11^a	1.10 ± 0.14^a
Condition factor	1.56 ± 0.11^a	1.00 ± 0.22^b	0.92 ± 0.18^b	0.76 ± 0.25^b

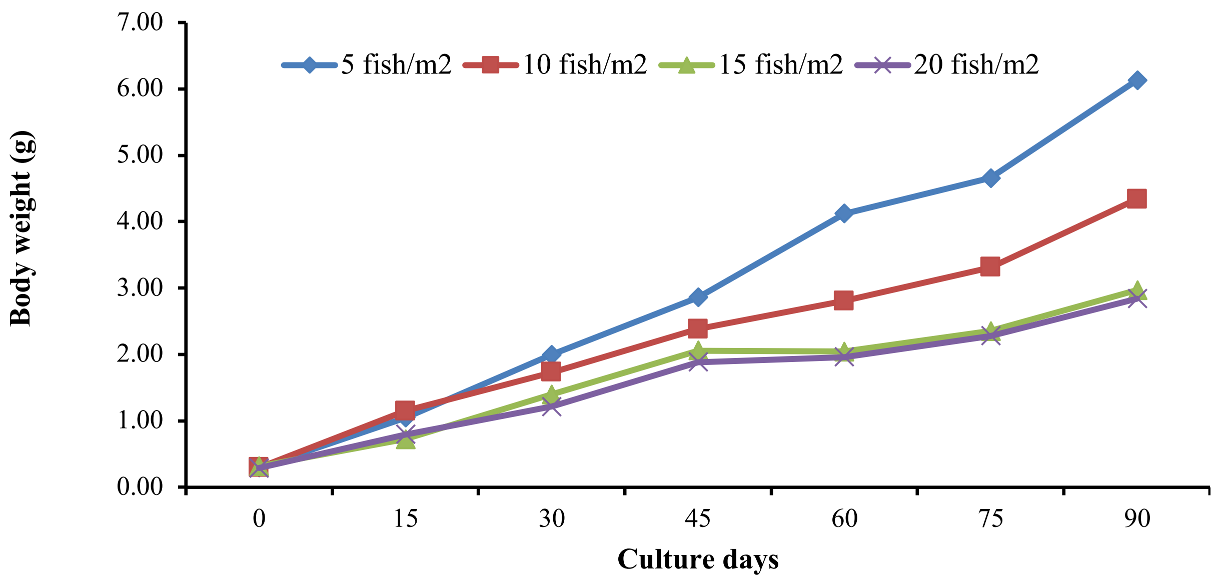

Figure 3: Size of fish over time in different treatments during the experimental period

Water temperature of the nursery pond ranged from 29.1-31.8°C during the experimental period, while mean dissolved oxygen was 7.7 (3.1-10.4), pH 8.4 (7.3-9.3) and Secchi disk depth was 30.5 cm (26.8-33.5).

Table 5. Mean and range of water quality parameters during experimental period

Parameter	Mean (Range)
Temperature (°C)	30.1 (29.1-31.8)
Dissolved oxygen (mg/L)	7.7 (3.1-10.4)
pH	8.4 (7.3-9.3)
Secchi disk depth (cm)	30.5 (26.8-33.5)

DISCUSSION

Sahar is known to be intermittent in spawning behavior in nature, and we found similar results in our rearing experiments. MacDonald (1948) was probably the first to mention that "…. Sahar is said to spawn three times in a season." Similarly, Desai (1994) noted breeding of sahar from July to March in a non-Himalayan River, Narmada, Madhya Pradesh, India. Pathani (1983) speculated there were at least four spawning acts on the basis of four different egg diameters and stages of development in mature ovary of mahseer (another name for sahar). Most findings indicate that sahar is a partial spawner due to the low number of eggs released during a single spawning event (Shrestha et al., 1990, Joshi, 1994, Shrestha, 1997). While partial or intermittent spawning characteristics may be important to produce more consistent overall reproduction in natural populations, they present a problem in using the fish for hatchery production. This can be overcome by regular inspection of the maturity status of females, but it is much more time consuming than using fish that spawn completely in one event and in synchrony. Partial spawning also results in fewer eggs from each spawn, limiting hatchery production of fry. Seed produced by artificial methods is important in helping to conserve and develop natural fisheries for this species, and successes of captive breeding are useful for aquaculture development. We demonstrated that mass seed production and larval rearing of sahar is possible in the subtropical region of Nepal by inducing maturation with a synthetic hormone. Induced spawning reduces the number of over-matured females found in captive populations by synchronizing the stripping time for injected fish.

Regular maturity observation at the correct spawning time is critical for sahar breeding, and thus, frequent examination of female fish for maturity is required. Low fecundity of this species, compared to other cultured carps, demands more female fish for mass seed production as well. As reported by Bista et al. (2010), pond reared sahar have intermittent spawning characteristics, and frequent checking of brood fish to determine optimum timing for egg stripping should result in a spawning success rate of more than 50%. Although we conducted frequent examination of brood fish for maturity, 6 females were found over mature, while successful breeding was attained by 6 females naturally and by 16 after hormone induction. Overripe females were recorded in our earlier study even when temperature ranged between 15.5-28.7°C, from the last week of November to the last week of February. Bista et al. (2010) reported that spawning occurred when temperature ranged between 26-27.4°C on one occasion, and 20-21°C on a second occasion in Pokhara, and there were more spawners in February-March compared to September-October. Pandey et al. (1998) reported successful spawning induced by hormonal injection when water temperature in the pond was 18-24 °C. However, the dose administered was lower (0.2 ml/kg body weight) than in this study. Bista et al. (2010) reported an incubation period of 45-125 hours at water temperatures from 19-28°C, with the shorter incubation period attributed to higher temperature. Bista et al. (2010) documented natural breeding in autumn when the temperature was 22-27°C, and in spring when the temperature was 19-25°C. Similarly, Pandey et al. (1998) reported induced breeding of sahar when temperature was 18-24°C.

Growth and survival rate of fry reared from artificial spawning were comparable to other studies on this species. Daily weight gain of sahar fry was considerably lower than reported by Paudel (2003; 0.10-0.13 g/day), Acharya (2004; 0.32 g/day) and Bista et al. (2008; 0.18-0.28 g/day) but higher than values recorded in Pokhara (0.02-0.03 g/day). Growth was comparable to fry reared in cooler and warmer parts of Nepal by Bista et al. (2008, Table 6). Growth appeared to be density dependent, with the highest growth occurring at density of 5 fish/m^2 and growth declining at higher densities. This is somewhat surprising because all of these densities were below the densities commonly used for rearing carp fry or sahar fry in Nepal (Table 6). High survival rate even with high density is a positive sign for fry rearing and mass seed production of sahar, but more work is needed to clarify the role of density in the growth of sahar fry.

Table 6. Survival and growth of sahar fry and yearlings reared in farm ponds from locations in the hills and Terai. Within a group (fry or fingerlings), values with different superscripts in the same row are significantly different.

	Fry		Yearlings	
	Hill	Terai	Hill	Terai
Stocking, No./m^2	20	20	1	1
Initial size, g	0.42	0.42	3.6±1.2 ^{a}	3.6±0.74 ^{a}
Growing days	90	90	210	210
Survival %	87.3±5.2 ^{a}	91.6±7.6 ^{a}		
Final weight, g	7.9±0.9 ^{a}	9.7±1.7 ^{b}	39.0±5.8 ^{a}	60.8±10.4 ^{b}
Growth/day g	0.087	0.107	0.18	0.28

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SPAT COLLECTION AND NURSERY METHODS FOR SHELLFISH CULTURE BY WOMEN

Quality Seedstock Development/Experiment/13QSD01PU

Narriman Jiddawi[1], Maria Haws[2], and Kwamena Quagrainie[3]

[1]*Institute of Marine Sciences (IMS), University of Dar es Salaam, Zanzibar, Tanzania*
[2]*Pacific Aquaculture and Coastal Resources Center, University of Hawaii-Hilo*
[3]*Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA*

ABSTRACT

Marine pearl culture in Zanzibar started in 2006. Currently, there is a growing interest in pearl culture among Tanzanian coastal communities as an alternative source of income. In order to make the activity sustainable, there was a need to find out where and when spat can be obtained instead of relying on adults. Thus, the objective of the current project was to develop best spat collection techniques and to determine the seasonality where spat can be obtained. The spat collection experiment produced a total of 3,354 *Pinctada margaritifera* spat and 3,861 *Pteria* spat at Bweleo and Nyamanzi respectively. The highest number of spat were observed from August to October 2016 and March to April 2017 for both sites. Three different types of spat collectors made of coconut shells, rubber tires, and black mesh were used during the study. There was significant variation on the number of spat produced from the three different spat collection materials ($P < 0.01$). Spat were most abundant on the new black mesh spat collectors than on coconut shells and rubber tires. Some of the spat captured from spat collectors were used for training purposes, specifically on how to culture and sustain such spat stocks. Based on the results of this study, it is possible to obtain pearl oyster spat and grow them in Zanzibar. The establishment of such an industry in Zanzibar would provide a much needed alternative livelihood among Tanzanian coastal communities, while utilizing coastal resources in sustainable way.

INTRODUCTION

Zanzibar has recently been the site for innovative work that combines development of aquaculture with integrated coastal management and fisheries management to implement alternative livelihoods. Zanzibar consists of two main islands and a number of small islands off of the east coast of Africa. The total area of both islands is 2,643 km^2 (Unguja is 1658 km^2 and Pemba is 985 km^2). The population is estimated to be around 1,300,000 people, which grows roughly 3.1% annually. Fishing is the most common coastal activity and is 95% of the fishing is artisanal, mostly operating in shallow water using traditional vessels and gear. However, the fisheries accessible to artisanal fishermen have been overfished (Jiddawi, 2012, Jiddawi and Ohman 2002), which has stressed local villages and the economy. Finding sustainable alternatives to fishing are a high priority of the national and local governments.

The residents of Zanzibar suffer from multiple nutrition and health issues related to poverty and marginalization. Of children under five years of age, 35% are stunted, 25% are underweight and 6% are wasted, resulting in approximately 130 child deaths per day (Jiddawi and Lindstrom, 2012, ZPRP 2002). This is one of the highest rates within Tanzania. Bivalve shellfish are good sources of protein, vitamins (C, B1, B2, B3, D) and nutrients such as calcium, iron, copper, iodine, magnesium, zinc, manganese, and phosphorus. Hence, farming of bivalve shellfish represents an alternative to artisanal fishing, a direct means of improving nutrition through local consumption, as well as a means for women to earn income to support basic family needs.

This project built on eight years of efforts to develop a small-scale bivalve shellfish industry led by women in East Africa. These efforts have successfully resulted in over seven coastal villages engaging in some form of bivalve shellfish farming (Jiddawi, 2011; Jiddawi 2007). Coastal women have traditionally utilized reef-gleaning of bivalves, other invertebrates and small fish as one of their livelihoods, and as the principal source of high protein food (Frocklin et al. 2014; Crona et al., 2010). This traditional livelihood is threatened by 1) increasing populations; 2) migration of inland populations to the coast; 3) development for tourism, which frequently excludes villagers; 4) over-fishing; and 5) climate change.

This project has been modeled on the successful efforts to develop seaweed farming by women, which is now a major coastal industry on the East African coast. Many of the women shellfish farmers have also engaged in seaweed farming; hence, they have a basic knowledge of aquaculture. Shellfish farming has equal potential as previous seaweed farming efforts if technical support is provided to refine existing methods to increase the sustainability of such efforts. This project focused sustainable production of *Pinctada margaritifera* and *Pteria penguin.*

Pinctada margaritifera and *Pteria penguin* are marine bivalve mollusc in the family. Pteriidae. *P. margaritifera* is commonly known as the black-lip pearl oyster and *P. penguin* is commonly known as the penguin's wing oyster. Both are used for the production of cultured pearls, meaning that these species are a valuable resource to humans. Long-term collection of adult pearl oysters from the wild for pearl production is not environmental sustainable. Sustainable pearl production is possible through collection of wild pearl oyster spat using spat collectors. Spat collection occurs when any material designed to attract spat settlement is placed in the water and tended. Properly designed spat collectors also protect the small spat while they grow.

These efforts support food security both directly and indirectly. Bivalve shellfish are an important source of protein and micronutrients for women and children. They are commonly the only source of protein that this group can access on a daily basis. Shellfish are also one of the main sources of income for women in Zanzibar, and the industries of producing shell jewelry and half pearls has begun to significantly increase women’s incomes. It has been documented that Zanzibar women use income from these activities for children’s school fees, food, and clothing and to improve their housing (Crawford et al., 2010; Haws et al., 2010; Lange and Jiddawi, 2009). Hence, stabilizing and scaling up bivalve culture is a feasible approach to improve food security and income for coastal women and children.

OBJECTIVES

The aim of this project was to develop a small-scale bivalve shellfish culture industry in Zanzibar to increase food security and family income with women as the primary participants. Specifically, this work addresses one of the primary obstacles to further development of the small shellfish farms: how to obtain stock in a sustainable manner for farms. Spat collection is one of the most sustainable and cost-effective methods to obtain stock for shellfish farms; hence, methods were tested to 1) determine the best materials and timing for spat collectors; 2) test nursery methods to rear the collected spat; and 3) provide training to women on other shellfish farming methods beyond the nursery stage.

METHODS

Pilot studies in 2009-2010 assisted in locating several sites near the villages of Bweleo and Nyamanzi on the Fumba Peninsula of Zanzibar, where spat settlement rates on artificial collectors were relatively high. Bweleo and Nyamanzi were among the eight villages participating in previous bivalve and pearl culture development work and their residents were among the most active in the participatory research. Approximately 200 women on the Fumba Peninsula have participated and/or benefitted from past aquaculture development efforts. Similar activities have been done in Mafia and

Mtwara on the Eastern Coast of Tanzania and showed promising results. Thus improvement of spat collection methods could be expanded to have regional benefits (Jiddawi, 1997).

Spat collection experiments were conducted by establishing submerged long lines in two areas and deploying 50 spat collectors every month over a one year period. Different spat collection materials (coconut and rubber tires; Figure 1a) and a new type of spat collection material (black mesh) (Figure 1b) were tested. This allowed researchers to determine the best time of year to deploy collectors and which material resulted in the highest level of spat settlement. The latter is more complex than it may appear because some materials may result in juveniles detaching themselves or being more vulnerable to predation (Haws, 2002). The long lines used to support the spat collectors were 10 m in length and were supported by buoys. The spat collectors were at a depth of at least 1 m depth from the surface during the lowest low tide because it is very important to make sure that the spat collectors do not touch the seabed during low tide.

Figure 1a. Spat collectors (from left: new spat collector, rubber tire and coconut shells)

Figure 1b. New Spat collectors

Collectors were inspected two months after deployment to obtain an estimate of the number of juvenile bivalves which attached and to identify these by species. Four months after deployment, the collectors were removed from the water and all adhering bivalves counted and identified. The juveniles produced in this manner were then used in the nursery experiments. Data on water quality (temperature and salinity) were also collected at each experimental site. Women from the two communities participated in all aspects of this work and were trained in the technical details of spat collection and nursery rearing.

One trip per month was taken to each of the villages, Nyamanzi and Bweleo, involved in pearl farming. The aim was to monitor the pilot spat collectors. The spat seemed to be attaching quite well. Spat seem to attach more frequently in Nyamanzi than in Bweleo village. Researchers also checked to ensure that those involved in the project were cleaning off the antifouling organisms properly. One of the visits was taken to a previous workshop site to see if the area had some stakeholders still involved.

The work was conducted in Bweleo and Nyamanzi within Fumba Peninsula (Figure 2). Nyamanzi is located about 16 km South West of Zanzibar Island. Mean air temperatures range from 27 ºC to 31 ºC during the wet season and 25 ºC to 28 ºC in the dry season. Salinity ranges from 36 ppt in February to 41 ppt in September. Bweleo is located on the western side of Fumba Peninsula, on Unguja Island. The area is characterized by a rocky shore with mean surface water temperatures ranging from 30.5 ºC in January to 26 ºC in June. Salinity ranges from 36 ppt in January to 40 ppt in July. Near-shore waters are generally sheltered from strong waves because of its location on the leeward side of the peninsula.

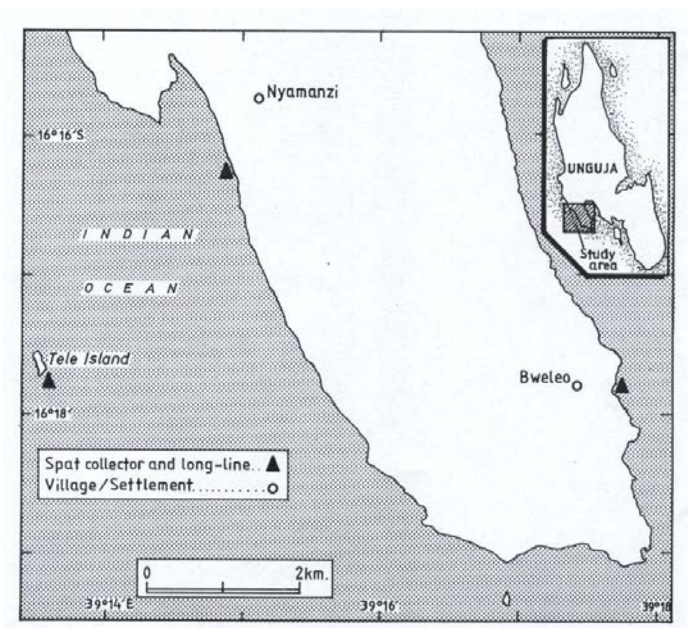


Figure 2. Study Area

RESULTS

The spat collection experiment involving women was conducted from August 2016 to June 2017 and produced a total of 3,354 *Pinctada margaritifera* spat and 3,861 *Pteria* spats at Bweleo and Nyamanzi respectively (Fig 3a and 3b). The spat collectors were hung in areas where there was the dense presence of adult pearl oysters in the surrounding waters to reliably produce high numbers of spat. The highest number of spat were observed from August to October 2016 and March to April 2017 for both sites. Previous results observed highest spat to be around the same periods of March to April as well as October to November (Jiddawi, 1995). Ishengoma (2011), however, observed highest catches in June. Based on the results of this short study, it is possible to obtain pearl oyster spat and grow them. More spat were found on the new black mesh spat collectors compared to the other two collectors. Mean water temperature were 26.15 ^{0}C at Bweleo and 26.35 ^{0}C at Nyamanzi. The salinity recorded in the both sites was the same with an average of 32 ppt.

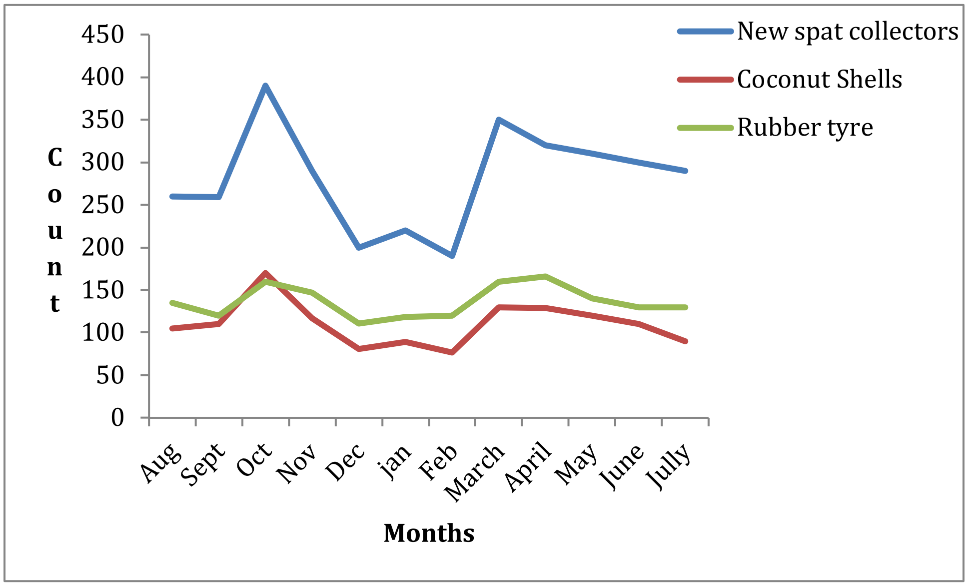


Figure 3a. Monthly number of *Pinctada* spat at Bweleo August 2016 to June 2017 on the various spat collectors.

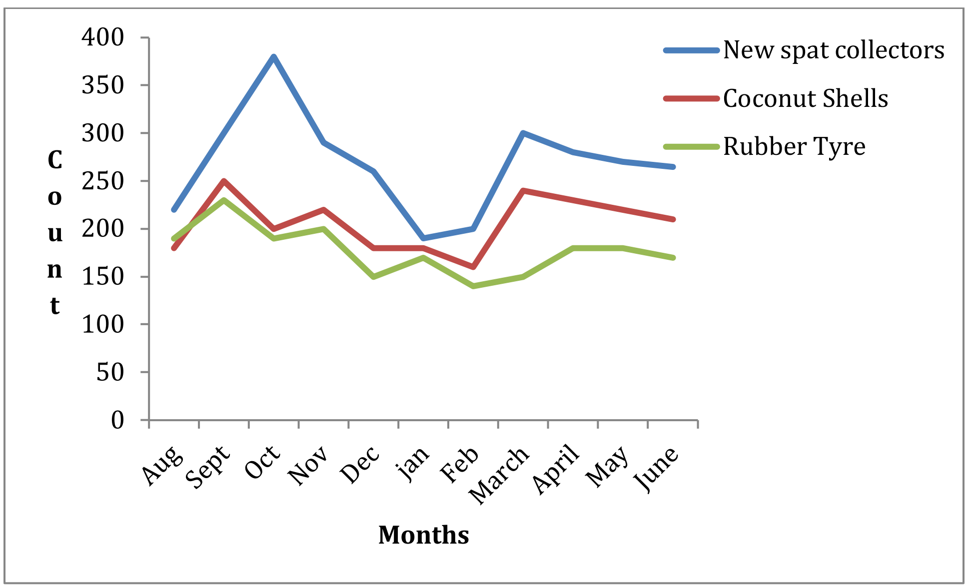
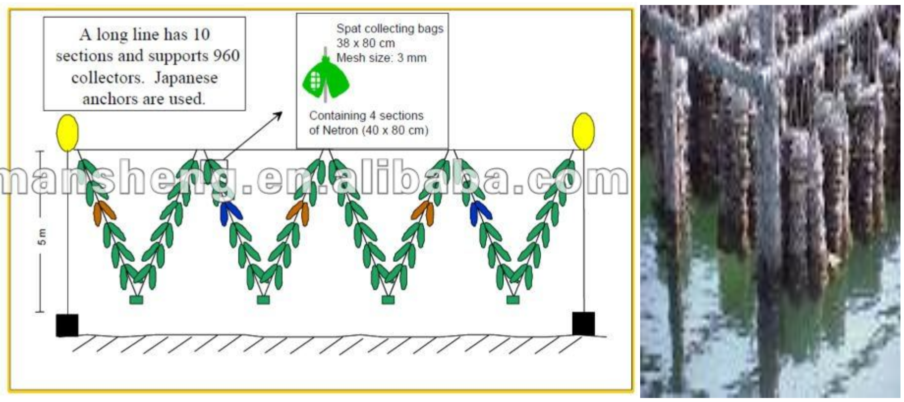


Figure 3b. Monthly number of Pteria spats at Nyamanzi August 2015 to March 2016 on the various spat collectors.

The women also participated in a one day training workshop on spat collection 20/11/2014 (Figure 4). This was done during the visit of Dr. Simon Elis, who visited Zanzibar in November 2014. He trained around 10 farmers from two Nyamanzi and Bweleo. The training focused on how to make spat collectors (Fig 4) and use them in an efficient manner. Swahili guidelines were printed and given to each participant. This will hopefully assist in improving oyster spat collection and pearl farming production techniques in Zanzibar. The manual elaborates on selection of collectors, when to install them and how to maintain them. After the meeting the participants were given some of the supplies purchased to make spat collectors when they return to their villages.



Underwater lines Spat collectors

Figure 4. Spat collectors used during training

DISCUSSION

It is important to select the correct type of material for spat collectors, choose the right areas for spat collection, place the spat collectors into the water at the right time of the year, and provide proper maintenance of the spat collectors and the long lines. In this experiment, the new black mesh collectors were found to be very efficient as they provided a greater number of areas for the oyster larvae to settle, grow, and avoid predation.

Also the local materials available like coconut shells, rubber tires can still be used although settlement on these materials is less than on the black mesh collectors.

From the follow-up trainings which were done with the communities on how to maintain, handle, and grow the spat to a size that they can use to seed the pearls using methods indicated by Haws (2002), the communities have welcomed this information because it relieves them from going out into deeper waters to collect the large shells and depending on men. These communities are also ready to train others to make this activity more sustainable and feasible by having enough shells to seed.

Overall at least eight community members who have been trained on using this new technique are very active and are ready to train others. Those involved in the training were also beach recorders of the Department of Fisheries, members from local NGOs and local communities. Also a training manual was produced of which there was one section is in Kiswahili (local language), which can be used efficiently.

Some problems were encountered:

- Fouling organisms, such as sponges and algae, can reduce the water circulation within a cage by blocking the nets' pores, which can then cause low supply of food and oxygen (hypoxia).
- High spat mortality during first month which continued to settle with high concentrations between October and November 2017.
- Some predators such as juvenile crabs, polychaetes, fishes and other marine organisms such as snails were observed.

CONCLUSIONS

Overall the project was very useful as the community learned a lot and it gave them a new insight on how to collect and handle spat and increased their overall aquaculture and marketing skills.

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Topic Area

HUMAN NUTRITION AND HUMAN HEALTH IMPACTS OF AQUACULTURE

❖

BETTER MANAGEMENT PRACTICES FOR MOLA-PRAWN-CARP GHER FARMING INTEGRATED WITH POND DYKE CROPPING FOR INCREASED HOUSEHOLD NUTRITION AND EARNINGS OF RURAL FARMERS IN SOUTHWEST BANGLADESH

Human Nutrition and Human Health Impacts of Aquaculture/Experiment/16HHI01NC

K.A. Huq[1], M. Ashraful Islam[2], N. Jahan[2], A. Haque[1], S. Saiful Islam[1], J. Bir[1], W. Sabbir[1], M. Abdul Wahab[3], S. Mahean Haque[3], C.A. Deck[4] and R.J. Borski[4]

[1]Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh
[2]Department of Horticulture, Bangladesh Agricultural University, Mymensingh, Bangladesh
[3]Department of Fisheries Management, Bangladesh Agriculture University, Mymensingh, Bangladesh
[4]Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA

ABSTRACT

In the Khulna region of Bangladesh, giant freshwater prawns are co-cultured with carps in paddy fields, a practice known as "*gher farming.*" The farmers, however, are typically unable to use sufficient amounts of quality feed due to the associated costs, limiting production and consequently household earnings and nutrition. We previously determined that incorporating Mola, a small indigenous fish species with high nutrient value, into prawn gher farming practices increased overall production and the farmer's income associated with the culture. Additionally, consumption of Mola was enhanced in families that incorporated it into their gher farming practice. The present investigation aimed to further improve the production of prawn, Mola, and carps through the addition of fertilizers to gher pond systems. In a second study, we also evaluated the effects of using these fertilized pond muds on the production of seasonal vegetables in integrated pond dyke systems to promote increased nutrition in rural Bangladeshi households. Three different types of fertilizer were applied monthly to ponds containing $2/m^2$ prawn, $0.1/m^2$ rohu, and $2/m^2$ Mola, in addition to a control treatment with no fertilizer. Treatments were as follows: T1, inorganic fertilizer, Urea 25 kg/ha + TSP 12.5 kg/ha; T2, organic fertilizer, molasses 30 kg/ha + yeast 0.3 kg/ha; T3, 50% T1 + 50% T2; and T4, no fertilizer. Prawn were raised in a nursery setting for 45 days before being transferred to the grow out pond. At the end of the 6-month grow out period, total prawn production was 459.12±14.6, 471.8±15.6, 508.8±13.94, and 396.8±17.8 kg/ha in T1-4, respectively. Mola and Rohu production exhibited similar trends, with fertilized ponds producing greater fish yields than unfertilized ponds and T3 having the greatest production overall. These results clearly indicate that the addition of fertilizers, particularly a combination of inorganic (Urea, TSP) and organic (molasses, yeast) to gher pond systems enhances the production of prawn as well as carps and Mola which will not only enhance incomes but provide additional food for the farmer's family to help increase household nutrition.

Muds excavated from the ponds used in Study 1 were then applied to pond dykes either alone or in combination with mulching to determine the best practice for integrating agriculture with aquaculture. The growth and yield of both summer (okra) and winter (tomato) vegetables were evaluated. Typically, plant growth and overall vegetable yield were greater for both tomato and okra grown in muds from fertilized ponds compared to unfertilized ponds. Further, incorporating mulching practices in addition to

the use of fertilized muds enhanced vegetable yield to an even greater extent, which could help to increase the vitamin and mineral intake in farming households. Together, these data indicate that the use of fertilizers in prawn-gher farming systems and the integration of these systems with agriculture practices can increase food availability and potential incomes and household nutrition for the families of prawn farmers by providing a greater abundance of marketable seafood as well as fish and vegetables for home consumption.

INTRODUCTION

The wide-spread use of integrated farming practices, including but not restricted to the production of multiple finfish, holds significant promise for increasing dietary nutrition, productivity, and profitability of farming households in rural Bangladesh (Lightfoot *et al*., 1990). Currently, rice and fish comprise the main diet of low-income families, particularly during the production season for these crops (Roos *et al*., 2007). Although integration of freshwater prawn (*Macrobrachium rosenbergii*) farming in seasonal rice/paddy fields (ghers) has been successfully implemented and serves as a significant source of income to coastal families, farmers typically sell the prawns produced to fetch higher prices in overseas markets. Meanwhile family members (particularly women and children) remain malnourished from lack of complete protein, vitamins, and other minerals in their diet. This investigation addressed this problem by incorporating additional crops [Mola fish (*Amblypharyngodon mola*) and vegetables] into current gher-prawn farming practices. As Mola fish and fresh vegetables are highly nutritional crops but have little potential for sale in cities or overseas markets, the cultivation of these foods will directly benefit local dietary needs. These studies tested novel integrated designs targeted specifically for increasing nutrition in low-income farming households of rural Bangladesh.

Child malnutrition continues to be a major public health problem in rural Bangladesh. Up to 38% of all pre-school children have vitamin A deficiency, with up to 55 percent exhibiting signs of iron-deficient anemia (Micronutrient Initiative/UNICEF, 2004; West, 2002). These effects may be alleviated, in part, through consumption of small indigenous fishes, such as Mola, which have significantly higher concentrations of vitamin A (~1900 IU, Thilsted et al. 1997) and micronutrient content than other commonly consumed fishes (e.g. carp). The Mola, a fish from 12-15 cm in length with soft bones, is particularly favored in the diets of many people; however, consumption is limited to those captured in local rice fields, rivers, and canals. Early experiments suggest that Mola can be successfully cultivated in the presence of other finfish cultivars (e.g., carp; Alim *et al*., 2004; Wahab *et al*., 2003). These fish are self-recruiting species, existing naturally in perennial ponds and other freshwater sources. Once stocked, Mola can reproduce within the ghers or in drainage ponds and can be continuously harvested over the production cycle of carp or prawn allowing for home consumption. Mola feed primarily on phytoplankton and detritus, therefore no feed input is necessary. Moreover, their bacteria-enriched waste can be utilized to enhance prawn production.

In our previous Innovation Lab research, we found that Mola and Rohu carp (*Labeo rohita*) could be successfully incorporated into gher-pond freshwater prawn culture without effecting prawn yield. We actually found that prawn production was somewhat enhanced by the presence of Mola and Rohu in polyculture. More importantly, production of Mola and Rohu increased the consumption of these nutritional foods and provided additional income from sale at local markets by households who undertook the polyculture farming practice. The study suggested stocking 2 brood of Mola/m^2 in prawn-carp gher-farming systems was best for increasing production of the fish without affecting their growth or that of other fishes. Molasses (30 kg/ha) and yeast (400g/ha) were used to fertilize ponds and our observations suggest it may increase production of protein enriched biofloc on the pond bottom and better buffer changes in pH that may occur with other organic or even inorganic fertilizers (personal observations; D'Abramo et al. 2009; New et al. 2010). Farmers are interested in understanding if this would be the best method for increasing prawn and fish production compared with inorganic fertilizers, which are commonly used for enhancing plankton for fish production (Javed et al., 1993; Qin et al., 1995; Jasmine et al., 2011; for review see Egna and Boyd, 1997). Therefore, this study evaluated which method of fertilization; yeast/molasses, inorganic fertilizer, or the combination of the two would best promote production of prawn, carp, and Mola and yield the greatest return on investment.

The advantage of integration of aquaculture with agriculture (Aquatic-Agriculture System, AAS) is that the nutrient rich pond muds and water derived from fish culture systems can be used for growing vegetables on pond dykes. It is an increasing trend for farmers to use inorganic fertilizer in Bangladesh and the country faces a large fertilizer deficit. Consequently, the share of imported urea has increased from 30% in 2005–2006 to 69% in 2010–2011 and the country is almost completely dependent on imports of triple super phosphate (TSP) and muriate of potash (MP) (Ahmed, 2011). By 2050, the country's inorganic fertilizer requirements will be higher and international fertilizer markets are becoming increasingly more volatile (Basak et al., 2015). So, use of pond muds may reduce the costs of crop production and can also improve the pond water quality by reducing the possibility of eutrophication. Pond muds from carp and tilapia production have proved to be potential fertilizer for the cultivation of seasonal vegetables in Northern Bangladesh (Wahab et al., 2001), but this had yet to be applied to the Southern regions of Bangladesh where prawns are grown on over 50,000 hectares and where seasonal water bodies (2.83 million hectares; flooded for 4-6 months) remain underutilized in Bangladesh (Kunda et al., 2008; DOF 2012). Our recent Phase I project shows that pond mud yields higher production of two seasonal vegetables (gourd and spinach) over dyke soil alone. The better utilization of pond muds with mulching material like black polythene has the potential to further increase vegetable production and its benefit will be analyzed and compared with that of inorganic fertilizer in the proposed studies.

Among household members, women and children often suffer most from a lack of nutritious foods. One key element of this investigation, therefore will involve training of women and girls on nutrition and better management practices for producing foods. Their greatest contributions to farming are feeding and maintaining fish and growing vegetables (Belton et al., 2011; Apu, 2014). We will train women on how to conduct the studies and provide them with best practices for improving production of fishes and crops, namely Mola and vegetables, that can improve household consumption of nutritious foods and provide additional income.

OBJECTIVES

1. Investigate the effect of three different fertilizers on the production performance of prawn-Rohu-Mola in integrated gher farming systems
2. Assess the comparative efficiency of prawn-gher/pond muds, mulch, and inorganic fertilizer on production of summer and winter vegetables in integrated pond dyke systems

MATERIALS AND METHODS

Location

This investigation was carried out on participating farms located in villages of Rangpur Union, Dumuria Upazila, Khulna District, Bangladesh and the surrounding region. Water quality was analyzed at Khulna University and pond dyke soil and pond muds at Bangladesh Agricultural University (BAU) in Bangladesh.

Study Area

Location:
Dumuria Upazila,
Khulna District,

Southwest Bangladesh

Study 1: Effect of different fertilizers on production of prawn, carp, and Mola in traditional gher farming systems of rural farmers in Southwest Bangladesh

Pond Drying, Excavation, Liming and watering

All farmers dried their nursery and grow out ponds just after harvesting the rice paddy. As part of the pond preparation bottom mud from the nursery ponds was excavated and the grow-out ponds were tilled to remove decomposed organic matter. Once the ponds were completely dry and excess clay was excavated, the ponds were limed at a rate of 0.25 kg/m^2 with limestone ($CaCO_3$) for the nursery ponds. The grow-out ponds were also limed. Five to seven days after liming, the ponds were filled with underground and rain water to a depth of 1-1.5 m. The excavated ponds muds from this study was used in Study 2 as nutrient-rich fertilizer for dyke vegetables.

Feeding of nursery and grow-out ponds

During the nursery period prawn post-larvae were fed 3-4 times each day with a commercial nursery feed at a rate of 200 g/10000 prawn post-larvae that was increased by 50 g/day until day 45. After stocking in grow out pond, prawn were fed thrice in a week following the farmers practice in their traditional gher farming. No supplementary feed was used to raise Mola which fed on natural foods resulting from pond fertilization.

Experimental design

Ponds were stocked with prawn, Rohu, and Mola at densities described in Table 1 and then treated with one of three fertilizers (T1-3) in an attempt to increase natural feeds within the systems or left unfertilized (T4). The first treatment (T1) incorporated inorganic fertilizers, adding 25 kg N/ha in the form of urea and 12.5 kg P/ha as triple super phosphate (TSP); the second treatment (T2) used a fermented mixture of 30 kg/ha molasses and 0.30 kg/ha powdered yeast; the third treatment (T3) consisted of a combination of 50% the amount of T1 and 50% the amount of T2 (Table 1). Fertilizers were applied monthly and each treatment group was replicated 5 times.

Table 1. Experimental design for Study 1.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Prawns (*M. rosenbergii*)	$2/m^2$	$2/m^2$	$2/m^2$	$2/m^2$
Rohu (*L. rohita*)	$0.1/ m^2$	$0.1/m^2$	$0.1/m^2$	$0.1/m^2$
Mola (*A. mola*)	$2/m^2$	$2/m^2$	$2/m^2$	$2/m^2$
Fertilizer (kg/ha)	Urea (25) + TSP (12.5)	Molasses (30) + Yeast (0.3)	50 % of T1 + 50% of T2	N/A

Water quality parameters

Temperature, transparency, DO, pH, alkalinity, ammonia, nitrates, phosphates, and chlorophyll-a were measured monthly. A secchi-disk was used to measure transparency. Alkalinity, ammonia, nitrates, and phosphates were measured using a HACH kit (HACH, USA; Model FF-2).

Growth Sampling

Monthly sampling assessed the health and measured growth of prawn, Mola, and Rohu.

Statistical Analysis

Differences between groups were assessed by one-way ANOVA with a Tukey's HSD post hoc test ($p < 0.05$) using Minitab Version 16 (Minitab Inc., State College, PA, USA).

Fish Consumption

Individual farmers recorded the amount of fish consumed by their households throughout the experimental period. Although our experimental period was six months, household fish consumption was determined annually as households consumed Mola from ponds during the study after the first reproductive cycle was completed and harvested regularly from the canal of the gher when rice was cultivated.

Study 2 - Assess the comparative efficiency of prawn gher/pond muds, mulch, and inorganic fertilizer on production of summer and winter vegetables in integrated pond dyke systems

Experimental design

The experiments consisted of nine treatment combinations employing pond sediments from the different fertilizer treatments used in Study 1 either alone or in combination with mulch and inorganic fertilizer (Table 2). Fertilizer was applied monthly to the ponds prior to being added to the vegetable plots and each treatment was replicated in 3 separate pond dykes putting the total number of plots per dyke at 9. Individual plots measured 1.5 m x 1.5 m and all plots within a dyke were separated by a 50 cm gap. Two separate trials were performed, one in winter using tomato and one in summer using okra. Tomato seedlings were allowed to grow for 25 days before being transplanted into the pond dykes in December while okra seeds were sown directly into the dykes in March. Mulching for selected plots occurred 15 days post-transplant for tomato and 15 days post-germination for okra.

Table 2. Experimental design for Study 2. Vegetables were grown in pond dykes used for fish grow-out and using pond muds from the different fertilizer treatments in Study 1.

Pond Mud (Study 1)	Soil Treatment 1	Soil Treatment 2	Soil Treatment 3
P1 (Urea + TSP)	Pond mud	Pond mud + mulch	Pond mud + mulch + inorganic fertilizer
P2 (Molasses + Yeast)	Pond mud	Pond mud + mulch	Pond mud + mulch + inorganic fertilizer
P3 (No Fertilizer)	Pond mud	Pond mud + mulch	Pond mud + mulch + inorganic fertilizer

Growth Sampling

For tomato, plant height and number of leaves per plant were measured at 30, 45, and 60 days while fruit weight and total yield were measured at the end of the 60-day experimental period. For okra, plant height was measured at 30, 45, and 60 days while number of flowers, number of pods, pod weight, and total yield were measured at the end of the experiment.

Statistical analysis

Differences between groups were assessed by one-way ANOVA and Tukey's HSD post-hoc test ($p < 0.05$) using Minitab Version 16 (Minitab Inc., State College, PA, USA).

Training and Dissemination

Farmers selected to participate in the study were informed of the nutritional value of Mola and the potential benefits of incorporating this species into existing prawn cultures and received hands-on training for techniques such as pond preparation, liming, fertilizing, feeding, post-larvae nursing, fish marketing, dyke vegetable culture, and disease control. We also held 4 training sessions from October 2017 to January 2018 in which 100 additional fish farmers (63 female, 37 male) were informed about protocols for pond fertilization and integrating prawn-carp-Mola gher farming with dyke cropping. Hands-on training was provided to the participants and leaflets outlining the findings from our previous Innovation Lab studies were distributed among them. At the end of January, additional training sessions were arranged and in attendance were prawn-carp gher farmers, local government authorities, school teachers, village leaders, and health workers.

Workshop

As part of Investigation 16MNE02NC the AquaFish Innovation Lab research team from Khulna University organized an interactive workshop on December 21, 2017 entitled "*Integration of Nutrient-rich small fish and vegetables with Prawn-Carp Gher farming in Southwest Bangladesh*" to help disseminate the outcomes from our prior studies to various organizations and the local farming community. More than 50 attendees consisting of representatives from Department of Fisheries (DoF), Bangladesh Fisheries Research Institute (BFRI), Department of Agriculture Extension (DAE), Soil Resource Development Institute (SRDI), various development NGOs (WorldFish, SOLIDARIDAD, Blue Gold, Winrock International, Shushilan, Rupantor, Prodipon, Nobolok, Ashroy Foundation), faculty from various universitites and local gher farmers were present at the workshop.We published the information provided in the workshop in six different Bangladeshi newpapers and leaflets on the research findings were distributed to the participants. As the Department of Fisheries is the government organization responsible for disseminating fisheries-related technologies to local farmers, the leaflets were also distributed to officers at various levels within the department, e.g. Deputy Director, District Fisheries Officer, Upazilla Fisheries Officers of Khulna Division. We believe that through these sectors that thousands of farmers were informed of these new technologies.

RESULTS AND DISCUSSION

Study 1 - Effect of different fertilizers on production of prawn, carp, and Mola in traditional gher farming systems of rural farmers in Southwest Bangladesh

Water quality parameters (Table 3) and total production for all species (Table 4) were measured monthly throughout the study period. Water quality was deemed suitable throughout the study period and there were no major differences between the four treatments with the exception of chlorophyll-a. This pigment provides the light energy necessary for plants to photosynthesize and the levels were significantly greater in fertilized ponds than in unfertilized ponds, suggesting an increase in natural food sources and overall pond productivity. No differences in prawn yields were observed between T1 and T2, however T3 exhibited significantly higher production relative to all other treatments. Further, total production of all species was greater in fertilized ponds (T1-3) than unfertilized ponds (T4) regardless of treatment, with T3 producing the largest yield overall (Table 4). This is likely due to an increase in natural food sources within the pond as a result of the added nutrients from the fertilizers. We also analyzed the ponds for phytoplankton and zooplankton abundance (Table 5) as this is the primary food source for Mola and carp. Consistent with the other parameters evaluated, fertilized ponds had a greater abundance of plankton than unfertilized ponds with the treatment combining both organic and inorganic fertilizers (T3) yielding the highest abundance overall. Lastly, the amount of fish farming families were able to consume in a year given the total production in each treatment was evaluated (Table 6). There were no differences in prawn or Rohu consumption as these species are more marketable and can be sold to enhance incomes. However, Mola consumption was greatest in T3 and higher in fertilized ponds than unfertilized ponds indicating that the use of fertilizers in gher pond systems can increase food availability for farming households.

Study 2 - Assess the comparative efficiency of prawn gher/pond muds, mulch, and inorganic fertilizer on production of summer and winter vegetables in integrated pond dyke systems

As inorganic fertilizers come at a substantial cost in Bangladesh due to the recent deficits and reliance on imports, we sought to determine whether fertilized pond muds and mulching could mitigate the need for fertilizers in integrated Aquatic-Agriculture Systems. Plant growth and yields are shown in Table 7 for tomato and Table 8 for okra. For both vegetables, plant growth parameters as well as overall vegetable yield were typically greater in plots containing mud from fertilized ponds (P1 and P2) than those containing mud from unfertilized ponds (P3), regardless of soil treatment. However, these parameters tended to be greater when combined with the addition of mulch and inorganic fertilizers rather than using the pond muds alone. Thus, the greatest overall yield for both vegetables was observed when mud from P1 was used in combination with T3 (Figures 1, 2). These data indicate that combining the use of fertilized pond muds with mulching practices in integrated Aquatic-Agriculture Systems could enhance crop production and provide additional food sources and nutrients to rural farming households in Bangladesh.

Training and Dissemination

Over 100 farmers were trained on protocols for integrating prawn-carp-Mola gher farming and dyke cropping with more than 50% of the participants being female. Women are primarily responsible for nutrition in farming households and also contribute to gher farming by feeding fish and cultivating dyke vegetables. Local fish farmers have been inspired to incorporate Mola fish and dyke vegetables into their cultures by observing the farming systems of those selected for the initial study trials and some have already begun this integration into their own traditional prawn-gher pond systems. These training sessions are thus helping to increase household nutrition and family incomes of a greater number of rural fish farmers.

Impact of Mola and vegetable dyke cropping with prawn-carp gher-polyculture
Workshop

The information disseminated during the workshop held at Khulna University was reported in 6 daily newpapers, on the university website (http://ku.ac.bd/latest-news/খুবিতে-গবেষণায়-গলদা-ও-রুই/), and in Farmer's Magazine (*Krishi kotha*; 75,000 copies). Leaflets from the workshop were distributed to officers at various levels within the Bangladesh Department of Fisheries to be distributed to rural farmers. We believe that through these sectors, thousands of farmers and other stakeholders will be informed of the new technologies so that the integration of Mola and dyke cropping on traditional prawn gher farms can be expanded throughout Southern Bangladesh.

CONCLUSION

The use of organic and inorganic fertilizers in prawn-gher systems enhanced production of all three species (prawn, Rohu, and Mola) by increasing natural feeds (phytoplankton) within the pond. This allowed for increased consumption of the nutrient-rich Mola by farming families as well as enhanced household earnings through greater market sales of prawn and Rohu carps. Additionally, the potential to use pond muds from these cultures as fertilizer for producing vegetables will help to mitigate the growing costs of agriculture due to the country's current fertilizer deficit. Further, the consumption of these vegetables will also aid in boosting household nutrition for these rural farming families by providing a greater diversity of vitamins and minerals, particularly for the women and children who are often malnourished.

QUANTIFIED ANTICIPATED BENEFITS

1. Increased production of prawn, Rohu, and Mola by the addition of organic and inorganic fertilizers to gher farming systems has been demonstrated in Southern Bangladesh.
2. Consumption of all three species, particularly the nutrient-rich Mola, has increased in farming households selected to test these new culture practices.
3. We have established that the use of fertilized pond muds from gher farming systems for growing tomato and okra in pond dykes in combination with mulching practices enhances plant growth and vegetable yield.
4. The integration of vegetable farming on "prawn-Mola" pond dykes offers great potential for the use of fertilized pond muds which could alleviate increased agriculture costs arising from the country's fertilizer deficit.
5. Introduction of Aquatic Agricultural System (AAS) has enriched the nutrient intake of women and children in prawn farming households.
6. The results of the study have been disseminated directly and indirectly to the thousands of farmers. Additionally, 63 women and girls have received on-site instruction on the nutritional and economic benefits of integrated farming designs and management practices for producing nutrient-rich fish and vegetables.

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TABLES AND FIGURES

Table 3. Water quality parameters measured in gher ponds treated with different fertilizers in Study 1. Values are means ± SD.

	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
Temperature (ºC)	31.98±1.68	31.86±2.21	31.46±2.42	31.56±2.35
Transparency (cm)	26.2±5.45	27.2±4.66	27.0±7.63	26.8±4.53
pH	7.38±0.29	7.32±0.23	7.3±0.18	7.35±0.2
DO (mg/L)	5.68±0.51	5.22±0.36	5.76±0.84	5.45±0.62
Alkalinity (mg/L)	200±50	170±27.38	190±41.83	202±24.25
Ammonia (NH_3-N mg/L)	0.33±.03	0.34±.021	0.35±0.03	0.28±0.02
Nitrate Nitrogen (NO_3-N mg/L)	2.99±0.93	3.46±0.94	3.86±1.12	2.97±1.02
Phosphate phosphorus (PO_4-P mg/L)	0.48±0.23	0.59±0.32	0.47±0.23	0.45±0.21
Chlorophyll-a (µg/L)	99.17±2.18	89.57±3.15	101.90±3.14	53.78±1.73*

Table 4. Production (kg/ha) of prawn, Mola, and Rohu in fertilized gher ponds from Study 1. Data are means ± SD. Values with different letters are significantly different (Tukey's HSD; $p < 0.05$).

	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
Prawn (kg/ha)	459.12 ± 14.6^{a}	471.8 ± 15.6^{a}	508.8 ± 13.9^{b}	396.8 ± 17.8^{c}
Mola (kg/ha)	608 ± 16.47^{ab}	589 ± 17.3^{a}	633 ± 15.4^{b}	547.6 ± 16.8^{c}
Rohu (kg/ha)	401 ± 11.6^{a}	417 ± 15.3^{ab}	440.4 ± 18.4^{b}	348.4 ± 23.3^{c}
Total (kg/ha)	1468.12±91.28	1477.80±75.67	1582.2±83.93	1292.8±90.12

Table 5. Phytoplankton and zooplankton abundance in gher ponds treated with different fertilizers from Study 1. Values are means ± SD.

	Phytoplankton No. /ml	**Zooplankton No. /ml**
Treatment 1	$6.83 \pm 1.67 x10^{3}$	$0.52 \pm 0.07\ x10^{3}$
Treatment 2	$6.19 \pm 1.48\ x10^{3}$	$0.64 \pm 0.06\ x10^{3}$
Treatment 3	$7.63 \pm 0.45\ x10^{3}$	$0.73 \pm 0.44\ x10^{3}$
Treatment 4	$4.16 \pm 1.05\ x10^{3}$	$0.42 \pm 0.09\ x10^{3}$

Table 6. Annual consumption of prawn, Mola, and Rohu by farming households in Study 1. Data are means ± SD. Values with different letters are significantly different (Tukey's HSD; $p < 0.05$).

	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
Prawn (kg)	16.93±1.1	24.2±0.5	32.87±0.6	22.98±0.8
Rohu (kg)	142.25±4.0	139.69±2.9	153±2.8	127±8.9
Mola (kg)	108.7 ± 2.15^{ab}	113.5 ± 1.6^{b}	121.6 ± 2.0^{b}	102.3 ± 16.9^{a}
Total (kg)	257.88±27.45	277.39±32.41	307.47±23.37	252.28±42.34

Table 7. Effect of pond muds and horticultural practices on the growth and yield of tomato. Values are mean ± SEM. Different letters indicate significant differences (Tukey's HSD; $p<0.05$).

	Plant height (cm)			Number of leaves						
	30 Days	45 Days	60 Days	30 Days	45 Days	60 Days	flower clusters per plant	Fruit Weight (g)	Yield (kg/plant)	Yield (kg/plot)
P_1T_1	58.5 ± 1.0^{b}	69.3 ± 0.8^{b}	75.8 ± 0.6^{b}	57.8 ± 1.1^{b}	66.2 ± 1.2^{b}	73.1 ± 1.3^{b}	7.0 ± 0.2^{a}	95.5 ± 2.5^{a}	3.3 ± 0.1^{c}	28.5 ± 0.3^{c}
P_1T_2	59.9 ± 1.7^{b}	71.0 ± 1.25^{b}	78.6 ± 0.8^{b}	61.27 ± 2.5^{b}	68.5 ± 2.6^{b}	75.5 ± 2.5^{b}	7.1 ± 0.1^{a}	100.3 ± 6.2^{a}	3.8 ± 0.05^{a}	30.2 ± 0.4^{b}
P_1T_3	66.7 ± 1.3^{a}	78.5 ± 1.37^{a}	85.2 ± 1.2^{a}	70.80 ± 1.9^{a}	77.6 ± 1.9^{a}	85.1 ± 1.0^{a}	7.5 ± 0.2^{a}	107.1 ± 4.3^{a}	4.0 ± 0.12^{a}	32.3 ± 0.4^{a}
P_2T_1	56.3 ± 1.1^{b}	66.7 ± 1.4^{c}	73.1 ± 1.4^{c}	59.2 ± 0.9^{b}	65.7 ± 0.5^{b}	72.3 ± 0.3^{c}	6.9 ± 0.1^{b}	98.2 ± 0.8^{a}	3.6 ± 0.08^{b}	28.5 ± 0.3^{c}
P_2T_2	57.1 ± 0.5^{b}	67.7 ± 0.8^{c}	73.2 ± 1.0^{c}	60.2 ± 0.1^{b}	67.9 ± 0.3^{b}	75.2 ± 0.4^{b}	6.9 ± 0.1^{a}	101.1 ± 1.5^{a}	3.8 ± 0.04^{a}	29.5 ± 0.4^{b}
P_2T_3	60.8 ± 1.5^{b}	72.9 ± 1.0^{b}	80.1 ± 1.4^{b}	64.1 ± 0.8^{b}	71.4 ± 0.8^{b}	78.1 ± 0.6^{b}	7.6 ± 0.1^{a}	106.8 ± 1.1^{a}	4.0 ± 0.8^{a}	30.3 ± 0.4^{b}
P_3T_1	49.7 ± 0.8^{d}	58.5 ± 0.7^{d}	64.5 ± 0.9^{d}	52.9 ± 1.19^{c}	60.5 ± 0.9^{c}	66.9 ± 0.9^{d}	6.5 ± 0.1^{b}	78.9 ± 3.7^{a}	2.6 ± 0.11^{f}	21.8 ± 0.4^{e}
P_3T_2	50.8 ± 1.2^{c}	61.1 ± 0.9^{d}	68.1 ± 0.4^{d}	52.3 ± 0.39^{d}	59.9 ± 0.5^{d}	66.9 ± 0.3^{d}	6.4 ± 0.1^{b}	96.4 ± 1.0^{a}	3.1 ± 0.03^{e}	24.5 ± 0.3^{d}
P_3T_3	47.0 ± 1.1^{d}	54.3 ± 0.9^{e}	61.7 ± 0.4^{e}	49.1 ± 0.4^{e}	55.9 ± 0.4^{e}	62.0 ± 0.6^{e}	5.5 ± 0.1^{c}	91.3 ± 2.5^{a}	3.1 ± 0.1^{d}	24.7 ± 0.3^{d}

Table 8. Effect of pond muds and horticultural practices on the growth and yield of okra. Values are means ± SEM. Different letters indicate significant differences (Tukey's HSD; $p<0.05$).

	Plant height (cm)							
	30 Days	**45 Days**	**60 Days**	**No. of flowers per plant**	**No. of pods per plant**	**Pod weight (g)**	**Yield (kg/plant)**	**Yield (kg/plot)**
P_1T_1	45.7 ± 1.2^b	68.8 ± 1.0^b	89.5 ± 1.0^b	13.2 ± 1.0^a	12.9 ± 0.4^a	23.9 ± 0.7^a	0.31 ± 0.01^b	2.7 ± 0.1^b
P_1T_2	47.5 ± 0.8^a	75.3 ± 1.1^a	96.0 ± 1.2^a	13.5 ± 0.35^a	13.1 ± 0.4^a	23.9 ± 0.7^a	0.31 ± 0.01^b	2.7 ± 0.1^b
P_1T_3	50.3 ± 1.1^a	78.7 ± 1.3^a	100.4 ± 1.4^a	14.7 ± 0.4^a	14.3 ± 0.4^a	24.7 ± 0.7^a	0.35 ± 0.01^a	3.1 ± 0.3^a
P_2T_1	45.3 ± 1.1^b	74.7 ± 1.3^a	95.9 ± 1.4^a	13.7 ± 0.4^a	13.3 ± 0.4^a	22.7 ± 0.4^a	0.30 ± 0.01^b	2.7 ± 0.3^c
P_2T_2	48.3 ± 1.2^a	78.7 ± 0.9^a	99.8 ± 1.1^a	13.4 ± 0.4^a	13.1 ± 0.4^a	23.3 ± 0.7^a	0.30 ± 0.01^b	2.7 ± 0.3^c
P_2T_3	49.3 ± 1.1^a	78.0 ± 1.1^a	98.7 ± 1.3^a	14.3 ± 0.3^a	13.7 ± 0.3^a	24.9 ± 0.7^a	0.34 ± 0.01^a	3.0 ± 0.06^a
P_3T_1	39.5 ± 0.9^d	63.1 ± 0.8^c	85.4 ± 1.1^b	12.9 ± 0.3^b	12.67 ± 0.3^b	23.3 ± 0.7^a	0.29 ± 0.01^c	2.6 ± 0.1^c
P_3T_2	39.5 ± 0.8^d	65.1 ± 1.1^b	86.5 ± 1.2^b	12.9 ± 0.4^b	12.73 ± 0.4^b	22.9 ± 0.5^a	0.29 ± 0.01^c	2.6 ± 0.1^c
P_3T_3	41.6 ± 0.8^c	66.0 ± 1.3^b	87.2 ± 1.4^b	14.2 ± 0.4^a	13.87 ± 0.3^a	22.6 ± 0.5^a	0.31 ± 0.01^a	2.8 ± 0.1^b

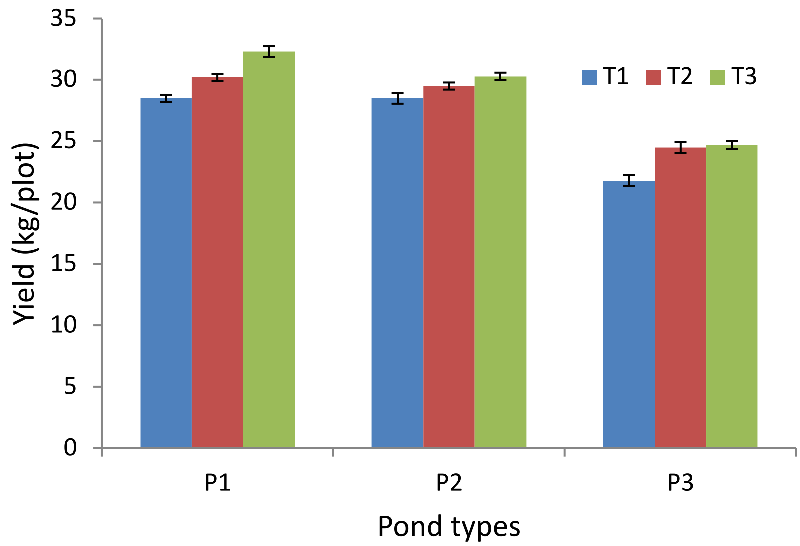

Figure 1. Effect of pond muds and agronomic management practices on the yield of tomato in Study 2. T1, 100% Pond mud; T2, 100% Pond mud + Mulch; T3, Pond mud + Mulch + Inorganic fertilizer. P1 (pond fish trial fertilization method), Urea + TSP; P2, Molasses + Yeast; P3, Unfertilized.

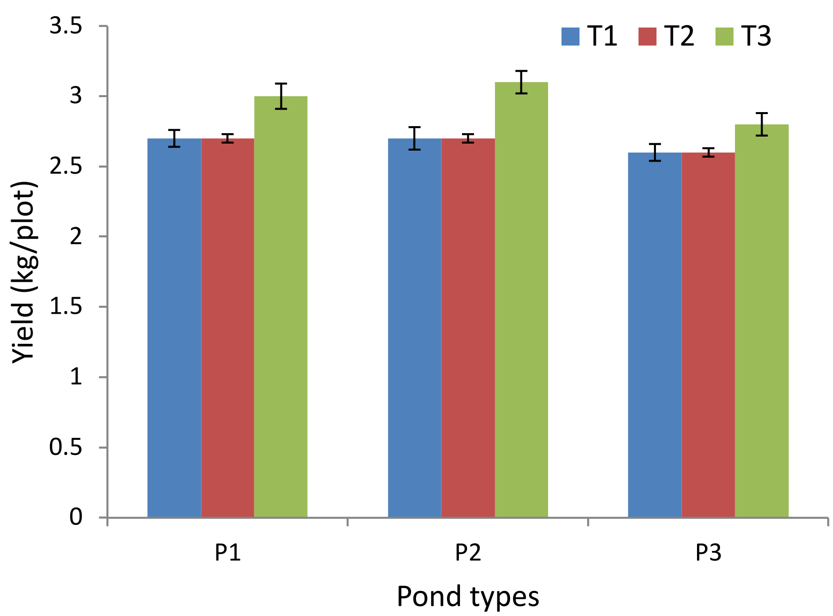

Figure 2. Effect of pond muds and agronomic management practices on the yield of okra in Study 2. T1, 100% Pond mud; T2, 100% Pond mud + Mulch; T3, Pond mud + Mulch + Inorganic fertilizer. P1, Urea + TSP; P2, Molasses + Yeast; P3, Unfertilized.

FISH CONSUMPTION AND IMPLICATIONS FOR HOUSEHOLD NUTRITION AND FOOD SECURITY IN TANZANIA AND GHANA

Human Nutrition and Human Health Impacts of Aquaculture/Study/16HHI02PU

Kwamena Quagrainie[1], Akua Akuffo[1], Sebastian Chenyambuga[2], and Steve Amisah[3]

[1]*Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA*
[2]*Sokoine University of Agriculture, Tanzania*
[3]*Kwame Nkrumah University of Science and Technology, Ghana*

ABSTRACT

Food security encapsulates the dimensions of food availability, food access, utilization and stability. While food availability emphasizes the presence of food in an appropriate quality and quantity, food access stresses the ability to acquire the amount of food necessary for a nutritious diet. Thus, food security of households depends to some extent on the diversity in the diet. The more diverse the diet, the greater the probability that the nutritional needs of households are being met. In terms of food access, under-provision of infrastructure and services enhances the remoteness of a location, which translates into inflated food costs. For example, poor roads increases transportation costs for goods and services to a locality, and combined with lack of telecommunication networks makes access to food expensive. Therefore, this study examined household food security improvements through fish farming and seafood consumption in Ghana and seafood accessibility in Tanzania. In Ghana, the study focused on dietary diversity and seafood consumption while in Tanzania, the study focused on the impact of infrastructural features on access to seafood by Tanzanian households.

The study in Ghana utilized the Food Consumption Score (FCS), a measure of dietary diversity as a proxy for food security. FCS involves the collection of information on food consumed by households and are weighted according the energy content of the food item. The results suggested that fish farming households have higher food diversity and frequency of food consumed than non-fish farming households through direct consumption, and to some extent through the income effect. In addition, households in the savannah zone, especially in the rural areas were found to have a higher probability of improving their food security status by engaging in fish farming. Also, a household's probability of attaining high food security increases with fish farming as an extra source of income assuming the household is engaged in other non-fish farming related ventures. Post estimation analysis showed that a household in the rural savannah ecological zone with a female household head engaged in fish farming, has a probability of 96% of being food secure. On the margin, the probability of adopting fish farming increased with wealth, location, ecological zone and household size but decreased with household income per capita.

Regarding seafood consumption, the study used a Latent Class Model (LCM) of structural heterogeneity to model demand. The results suggested that Ghanaian consumers fall into two classes, which we refer to as 'Conservative' households and 'Progressive' households. For Conservative households, fish and poultry are complementary goods while fish and red meat are substitutes. For Progressive households, fish and poultry are complementary goods while fish, red meat and pork are substitutes. The potential reasons for the substitutability of fish over other animal proteins may relate to economical, dietary diversity, health and nutrition related factors and taste. Price is a major concern for consumers in the rural and peri-urban areas, who tend to be more Conservative, while taste, dietary diversity, health and nutrition concerns pertain to urban consumers, who tend to be more Progressive. An increase in the number of years of education of Conservative households reduces the consumption of fish but education has no impact on fish consumption by Progressive households.

Conservative households are identified as being Akan Christians, and located in the forest and savannah areas while Progressive households are identified as a mixture of Akan, Ewe and Dagomba. Religion does not affect fish consumption by Progressive households, located in mainly the savannah areas. Based on these results, it is recommended that producers take advantage of lifestyles and belief systems to improve marketing of seafood in Ghana by adopting consumer targeting, market segmentation, and positioning strategies in marketing their fish.

In Tanzania, accessibility to seafood was evaluated through the impact of infrastructural features such as electricity, communication networks and transportation. Two measures of seafood accessibility were used, i.e., Fish Accessibility Count (FAC), which is simply the total count of outlets a household visited over the period of data collection to obtain fish; and a Food Accessibility Index (FAI), an index constructed from a household's FAC, weighted by the population and average accessibility count in the enumeration area. The regression results showed that access to seafood by rural, urban and peri-urban households is determined by different factors. Access to transportation is a significant determinant of access to seafood by urban households, access to electricity improve access to seafood by rural and peri-urban households while access to communication improve access to seafood by rural and urban households. Other significant determinants of access to seafood include price, household size, age, education of the caregiver, the caregiver being married, and employment. A comparison between the FAC and FAI estimates shows that the estimated coefficients from the FAI are smaller in magnitude than estimates from the FAC model. The outcome of this study highlights the importance of infrastructure in Tanzania to seafood accessibility, particularly electricity, communication and transportation.

INTRODUCTION

The concept of food security has evolved over the years with initial focus on the volume and stability of food supplies. However, in 2001, the FAO redefined food security as "… *a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life*" (FAOa, 2002). Thus, food security of households depends to some extent on the diversity in the diet. The more diverse the diet, the greater the probability that the nutritional needs of households are being met. Similarly, lack of infrastructure heighten food insecurity. Poor road systems, inefficient market distribution and infrastructure systems such as post-harvest storage systems and unpliable roads hinder accessibility to food including seafood in many developing countries.

Fish is an important contributor to food security in Ghana and Tanzania, especially when food security is defined beyond the confines of availability and accessibility to encompass the nutritional content of food. Fish is an important source of proteins, essential micronutrients and minerals in the diet of most African households but supply is low so is the consumption levels of fish. The sub-region has the lowest per capita fish consumption in the world, nevertheless, it is projected that, in order to maintain current levels of fish consumption in Africa, an additional 1.6 million tons of fish is needed (WorldFish, 2009). Several developmental interventions in Africa related to fish consumption, aquaculture, and capture fisheries have aimed at improving the nutritional status of households through direct dietary intake, production and increase in household income (Kawarazuka, 2010).

The nutritional impact pathways of fish could come from fish farming, where the household consumes fish harvested from their pond, and/or from other indirect ways such as selling the harvested fish to increase the food purchasing power (income effect) of the household to purchase nutritious foods. Several factors are critical to the nutritional decisions of household such as income, tastes, education, family size and composition and market price (Abdulai and Aubert, 2004). The income and price factors represent the purchasing power and availability of food in the house, tastes represent food preferences, education etc., family size and composition depict the per capita purchasing power and food availability.

Food security encapsulates the dimensions of food availability, food access, utilization and stability. While food availability emphasizes the presence of food in an appropriate quality and quantity, food access stresses individuals' ability to acquire the amount of food necessary for a nutritious diet (FAO, 2006). Thus, under-provision of infrastructure and services enhances the remoteness of a location, which translates into inflated food costs. Poor roads increase transportation costs for goods and services to a locality and this combined with lack of telecommunication networks makes access to food expensive. The lack of food market information is very important as it hinders trade between rural and urban food producers and traders. Communities poorly connected to major food marketing centers suffer from the risk of uncertainty surrounding food production and marketing (Llanto, 2012, Temu et al., 2005).

OBJECTIVES

1. Measure household food security in terms of dietary diversity using indicator measures in Ghana
2. Analyze household food security in terms of seafood accessibility using infrastructure features in Tanzania
3. Analyze the determinants of household consumption practices of various food types including fish in Ghana
4. Formulate policy measures to improve fish consumption and subsequently, improve household food security in Ghana and Tanzania.

This study examined household food security improvements through seafood consumption in Ghana and seafood accessibility in Tanzania. For Ghana, the study focused on dietary diversity and seafood consumption while for Tanzania, the study focused on the impact of infrastructure features on access to seafood by Tanzanian households.

METHODS

Data and Study Design - Ghana

This study adopted the Food Consumption Score (FCS) method to assess food security in the Propensity Score Matching (PSM) framework. Fish farming households were classified as the *treated* group while non-fish farming households were classified as the *control* group. FCS is a measure of dietary diversity and involves the collection of information of food consumed by households and weighted according the energy content of food item (WFP, 2009). The food groups include cereals, roots and tubers, vegetables, fruits, meat, offal, poultry, eggs, fish and seafood, pulses / legumes / nuts, milk and milk products, sugar / honey, oils / fat, and condiments / miscellaneous. Supplemental data used in the analysis included the 2012/2013 Ghana Living Standards Survey (GLSS) data published in August 2014. The GLSS data has information on a range of factors including the living conditions and well-being of households in Ghana, demographic characteristics of households, education, health, employment, housing conditions, household agriculture, household expenditures, income and their components, access to financial services, and assets. There is information on 16,772 households from all ten regions of Ghana in the dataset, but information on 4,011 household was used that included 144 fish farmers. The sample size was determined after using influence diagnostics to identify observations that influence the variance. Using *rstudent* thresholds, observations were dropped if their *rstudent* values were outside the range of $2 \geq r \leq -2$. The rstudent was used to identify variables of influence, which significantly affected the model fit. Normally, observations with rstudent larger than 2 in absolute value need attention because of their pull on the dependent variable (Boomsma, 2014; SAS, 1999).

The analysis on seafood consumption utilized a Latent Class Model (LCM). Data used for the analysis involved information on 2,641 households in the GLSS database. This sample size was established after recognizing missing data on fish consumption, market prices and education. A significant portion of the GLSS database (13,300) was for households that had not purchased any of the animal protein of interest for this study during the period of data collection. Households with missing data on education and fish expenditure were also dropped, resulting in our sample size of 2,185. The information about the sample used covered the community, household, and individual levels on demographics, socioeconomic factors, expenditure and market prices.

Propensity Score Matching (PSM)

PSM is a quasi-experimental approach that reduces the estimation bias in measuring the impact of a treatment (in this study, fish farming) on an outcome (in this study, food security proxied by FSC) with observational data. PSM involves two stages; the first is a logit regression with the treatment variable as the dependent variable to estimate the adoption decision as a function of household observable characteristics. Propensity scores for both the *treated* (fish farming) and *control* (non-fish farming) were generated using the predicted results from the logit regression. Using matching algorithms, the *treated* and *control* households were paired up. The second stagewas the determination of the impact of the fish farming adoption decision on the outcome variable (household food security). The impact of the adoption decision on the outcome variable is estimated by calculating the net impact of adopting fish farming on the household's food security (Baker, 2000). The basic set up for PSM was;

$$Y_1 = \beta_1 X + \varepsilon_1$$
$$Y_0 = \beta_0 X + \varepsilon_0 \qquad (1)$$

where Y_1 is the outcome variable for the *treated* group; Y_0 is the outcome variable for the *control* group; *X* is the vector of observed characteristics for both c*ontrol* and *treated* groups, ε_1 and ε_0 represent the error terms assumed to be exogenous of the vector of observed covariates.

The focus of the analysis was the correlation of participation in fish farming denoted by *F* and household food security. If a household is participating in fish farming ($F = 1$), the expected average outcome of food security is $E(Y_1|F = 1)$ and the counterfactual situation when the household is not participating in fish farming is $E(Y_0|F = 1)$. The counterfactual is unobservable, however the food security outcome of a non-fish farming household ($F = 0$), $E(Y_0|F = 0)$ was observed. Therefore, the Average Treatment on the Treated (ATT) was estimated as;

$$ATT = E(Y_1 - Y_0|F = 1)$$
$$= E(Y_1|F = 1) - E(Y_0|F = 1) \qquad (2)$$

The PSM used the observed mean of the food security outcome of non-fish farming households who are similar to the fish farming households in the observed characteristics, i.e., it uses $E(Y_0|F = 0)$ to estimate $E(Y_0|F = 1)$;

$$E(Y_0|F = 1) - E(Y_0|F = 0) = 0 \qquad (3)$$

Equation (3) ensures that there is no bias from self-selection in the ATT.

FCS as a Food Security Indicator

For FCS, a measure of dietary diversity to be a valid indicator of food security, it has to capture food accessibility, availability, utilization and stability (Kennedy et al. 2010; Hoddinott and Yohannes, 2002). Household per capita income and wealth index were used as food security indicators because the strength of correlation validates FCS as a proxy measure of food security. From Table 1, FCS is correlated at the 5% level with household income (0.036), wealth index squared (0.051) and per capita household income (0.04), indicating a strong validation.

Table 1. Correlation of FCS with Other Food Security Indicators

Variable	FCS	Wealth index squared	Per capita household income	Household income
FCS	1.000			
Wealth index squared	0.051*	1.000		
Per capita household income	0.040*	0.012	1.000	
Household income	0.036*	-0.050*	0.894*	1.000

Note: * indicates statistical significance at 5% level

The determinant variables used to assess the impact on food security include, ecological location; fish farming; household wealth; household per capita income; household size; and the educational level, age, marital status, employment status, and gender of household head.

The Latent Class Model (LCM)

To model household fish consumption, a household is assumed to maximize utility from the best combination of commodities, subject to time, resources and technology. To address heterogeneity among households in the sample, a form of cluster analysis was applied, specifically the LCM. The LCM assigns households into classes, which is determined through probabilities. This avoids bias and randomness in sample selection. The LCM analysis simultaneously models the demand function with households classified into different expenditure classes. Tastes and preferences are assumed to be homogenous within a class, but differ across the classes (Birol et al., 2011). The allocation of a household to a particular class is purely probabilistic and it is dependent on the household's characteristics. The prior probability of a household belonging to a particular class in the presence of household characteristics k_i is modeled as:

$$P[class\ c|k_i] = N[\beta_c' x_i, \sigma_c^2] = F_{ic} = \frac{\exp(\theta_c', k_i)}{\sum_{c=1}^{C} \exp(\theta_c', k_i)}, \theta_C = 0 \quad (4)$$

Since k_i contains variables, a class specific variables are normalized to zero to identify the model. The standard errors are then bootstrapped to take care of outliers in the data and enhance asymptotic inference of the results.

The variables used for the LCM analysis included Fish Expenditure for a period of 7 days measured in Ghana cedis, which is the dependent variable. It includes all forms of fish, fresh, dried, smoked, salted and canned. The independent variables include market prices in Ghana cedis (Ghc) for red meat (goat meat, mutton, and beef & canned beef) and poultry (chicken); demographic factors, i.e., years of education, marital status, monthly income (proxied with total household expenditure in Ghana cedis), being employed, and age of household head; geographical location, i.e., coastal, forest, savannah (Sudan Savannah, Guinea Savannah and Coastal savannah) and the Greater Accra Metropolitan Area (GAMA); and cultural and religious factors, i.e., ethnicity and religious affiliation. The categories for the ethnic groups are Akan, Ewe, Ga, Guan, Dagomba and foreigners, and the religious affiliations are traditional, Islamic and Christian religions.

Although the data is cross sectional, households were surveyed in different months from the 4th quarter of 2012 through the 3rd quarter of 2013. Quarters were therefore included to capture inflation as well as seasons and occasions such as the Ramadan for the Muslims and other seasonal festivities.

Data and Study Design - Tanzania

Data for the analysis are from the Tanzania 2011/12 Household Budget Survey (HBS). The data were collected with structured questionnaires that solicited information on household and community demographics, socioeconomic, individual and health issues. The database contains information on

10,186 households from all 19 regions of Tanzania Mainland. Tanzania is divided into smallest units by land area called enumeration areas (EA), which are the next lowest cluster level after household level. There are 400 EAs with 26 households in each EA. This study utilized information on 1,730 households with data pertaining to household and community infrastructure and seafood accessibility. The variable *education* had about 8,000 missing values. We did not want to assume that households with zeroes and missing values were the same, so we dropped the households with missing values but left those with zeroes.

To assess seafood accessibility, there is a need to develop a measure of accessibility. This was accomplished with two different measures; (1) Fish Accessibility Count (FAC), which is simply the total count of outlets a household visited over the period of data collection to obtain fish; (2) a Fish Accessibility Index (FAI), which captures the heterogeneous nature of the location of these households. FAI weights each household's accessibility count by the total population and average accessibility count of its EA.

Fish Accessibility Index (FAI) as Food Access Indicator

In the Tanzania HBS data, households disclosed the outlets where they obtained fish. These include market, permanent shop, street vendor, supermarket, department store, other household, other, own production, gift from neighbors, food aid and gathering. The most frequented outlets were the market, shop and other unknown source(s). Each household's FAI is created as follows:

$$\text{FAI} = \frac{T - m_i}{n - 1} \tag{5}$$

where T is the total accessibility count for each EA., m_i is the average accessibility count of an enumeration area and n is the total number of households in the sample. It is expected that a more urbanized area and densely populated area will have more food outlets than otherwise. This has been the observation in most studies particularly in developed countries.

To validate FAI as a measure of fish access, it must capture two main components of food access; physical access (distance to market, number of food outlets in a location) and economic access (household income, market price of fish, quantity of food purchased). The FAI as a valid measure of seafood accessibility must also be correlated but not perfectly with these measures of access. A perfect correlation implies FAI is just measuring exactly the other measure of access. However, a significant but not perfect correlation between FAI and other measures of access is indicative of the difference between FAI and the other measures of access. From Table 2, FAI is correlated at the 5% level with access count (0.44). This means that the physical aspect of access explains about 44% of FAI.

Table 2. Correlation of FAI with Other Food Access Indicators

Variable	FAC	Price	Quantity of fish	FAI
FAC	1.000			
Price	-0.096*	1.000		
Quantity of fish	-0.0214	0.317*	1.000	
FAI	0.443*	-0.023	-0.011	1.000

Note: * indicates 5% significance level

Two regressions were estimated with the FAC and with the FAI as the dependent variables. The regressions used control variables to capture both the observable and unobservable factors influencing seafood accessibility. The conceptual regression equation of the household seafood accessibility model is:

$$\ln(y_i) = \beta_0 + \beta_i x_i + \ln(s_i) + \mu_i \qquad i = 1, \ldots, n \tag{6}$$

where y_i is the dependent variable, seafood accessibility; x_1 to x_n are exogenous independent variables of age, married, mother's education, household size, geographical location, sex of caregiver, and employment status; and s_i represents household monthly income, price of seafood and expenditures on transportation, electricity and communication. ln (.) denotes an inverse Hyperbolic Sine Transformation (IHS) which is a log-like transformation that allows the zero and negative values in the observations. The transformation takes the form of $= \ln(d_i + (d_i^2 + \theta)^{0.5})$, where θ = 1, is employed as IHS (see examples in Bellemare et al., 2017; MacKinnon and Magee, 1990). Since the IHS is a log-like transformation, the coefficients on price and income can be interpreted as elasticities. To incorporate the heterogeneous nature of individual households, the variances for equation (6) is estimated with equation (7) below to obtain White's standard errors as heteroscedasticity-robust standard errors:

$$Var(\hat{\beta}) = (X'X)^{-1}X \sum X(X'X)^{-1} \quad (7)$$

These standard errors are asymptotic and so inferences can be made about the entire population in the presence of any kind of heteroscedasticity, including homoscedasticity (Greene, 2003).

The Tanzania HBS database has information on the expenditure on community features by the household and that is what is used as proxy measures for access to transportation, electricity and cell phone networks. Access to electricity is the household's monthly expenditure on electricity in Tanzanian shillings (TZS); Access to Communication Networks is the household's monthly expenditure on mobile phone and internet use in TZS; and Access to transportation is the monthly expenditure of the household on transportation in TZS.

RESULTS AND DISCUSSION - GHANA

In order to estimate the impact fish farming has on household food security, the ATT was calculated after matching, and the results are shown in Table 3. All matching algorithms show similar results that on average, adopting fish farming significantly increases food security of households as measured by the FCS. The average improvement in food security is between 13.9 and 15.5 points. This increase in food security can be translated food-wise / dietary diversity into consuming fish at least twice a week; roots/tuber or cereals, pulses and legumes once a week; fats and oils or sugar and sugar products at least once a week; and vegetables or fruits at least twice a week.

Table 3. Impact of Fish Farming Participation on Household Food Security

Variables	Matching Algorithm	Treatment	Control	ATT	BSE	t-stat	FFH	nFFH
FCS	NNM (1)	69.77	54.23	15.54	1.71	9.11	143	3867
	NNM (5)	69.77	54.28	15.54	1.44	10.78	143	3867
	KBM (0.03)	69.77	55.30	13.86	1.38	10.31	143	3867
	KBM (0.06)	69.50	55.64	13.86	1.34	10.35	143	3867

NNM = Nearest Neighbor Matching; KBM = Kernel Based Matching
BSE = Bootstrapped standard errors with 100 replications
FFH = Fish farming Household; nFFH = non-Fish Farming Household

The major pathway through which fish farming contributes to poverty alleviation and economic development in Ghana is the multiplier effect (Kassam, 2014). However, from the findings in this study, the effect is direct consumption. It can be concluded that a household that participates in fish farming has a higher probability of achieving higher food security because of increased access and availability.

The results from the matching procedures are shown in Table 4. The results show whether the algorithm used was able to balance the distribution of the important covariates in the *treated* and *control* groups. There was significant percentage reduction in biases as seen in Table 4. No significant differences are seen between fish farming and non-fish farming households for any of the covariates after matching.

Table 4. Tests for Selection Bias after Matching

Variables	Matched sample				
	Treated	Control	%Bias	Bias Reduced	t-test p-value
Wealth index squared	2.16	1.86	10.8	47.4	0.48
Wealth index	0.12	0.11	1.0	95.8	0.94
Education	7.76	6.76	15.7	47.4	0.18
Age	45.99	46.24	-2.1	82.8	0.88
Peri-urban	0.21	0.24	-8.2	79.5	0.57
Married	0.93	0.92	2.1	94.2	0.82
Employed	0.97	0.99	-10.3	22.9	0.41
Sex	0.92	0.93	-2.2	91.5	0.82
Location	2.62	2.57	7.8	80.4	0.50
Income/capita	63.09	56.01	1.8	96.2	0.61
Household size	7.69	8.36	-16.3	68.8	0.24

Effects of Ecological Zone and Female-Headed Households on Food Security

The FCS thresholds of *Poor* (0 - 21), *Borderline* (21.5 - 35) and *Acceptable* (above 35) were used as dependent variable for further analysis, and the probabilities of households belonging to any of these categories calculated. The majority of the sample were located in the rural area, so to assess how rural households can improve their food security status; the variables were analyzed using a representative household: Being a fish farmer, a household with an educated female head, and living in the rural savannah ecological zone. The interest in the savannah ecological zone is because the three Northern regions (Upper East, Upper West and Northern) have the highest prevalence of food insecurity (Table 5). In addition, it was observed from the analysis that moving away from the coast towards inland (forest and savannah ecological zones) increases the probability of adoption of fish farming.

Table 5. Food Consumption Scores by Region

Region	Min(FCS)	Max(FCS)
Western	32	84.5
Central	39	88
Greater Accra	60	88
Volta	31.5	89.5
Eastern	34.5	85
Ashanti	25	112.02
Brong Ahafo	17.30	109
Northern	33	71
Upper East	30.5	73.5
Upper West	27	73.5

The post estimation results show that a household in the rural savannah ecological zone with a female household head engaged in fish farming, has a higher probability of being food secure (96%) as shown in Table 6.

Table 6. Probability of Improving Food Security in Rural Savannah Zone for Female Fish Farmer Household Head

Variable	Predicted prob.
Poor	0.001* (0.00)
Borderline	0.036* (0.01)
Acceptable	0.963*** (0.01)
Observations	4,000

Standard errors in parentheses
*** represents p<0.01, ** p<0.05, * p<0.1
NOTE: ALL other regressors at their mean value

Effect of Income Diversification on Household Food Security

Income diversification is another way of improving household food security. It helps to reduce risks associated with the households' ability to access food. Therefore, the ability of the household to increase its probability of access to food may increase if household income sources are diversified. Three different scenarios were simulated with three different income sources: (1) Non-fish farming income only, (2) income from fish farming plus non-fish farming (diversified income), and (3) income from only fish farming. Results of these simulations are presented in Table 7. The results show that the probability of households increasing their food security status increases with a diversified income source (89%), with only income from fish farming (86%) and with non-fish income (85%). The probabilities of being food insecure are very low; 0.004 for food poor with no income from fish farming, 0.004 with income from fish farming and 0.003 with a diversified income source. All estimates are statistically significant at the 1% level. These outcomes imply that household's probability of attaining food security increases with fish farming as an extra source of income assuming the household is engaged in other non-fish farming related ventures.

Table 7. Predicted Probabilities of Achieving Higher Food Security with Income Diversification

Variables	Predicted prob.
Poor with non-fish income	0.004*** (0.01)
Poor with fish income	0.004*** (0.00)
Poor with diverse income	0.003*** (0.00)
Borderline with non-fish income	0.142*** (0.01)
Borderline with fish income	0.133*** (0.02)
Borderline with diverse income	0.112*** (0.01)
Acceptable with non-fish income	0.854*** (0.01)
Acceptable with fish income	0.863***(0.02)
Acceptable with diverse income	0.885*** (0.01)
Observations	4,000

Standard errors in parentheses
*** represents p<0.01, ** p<0.05, * p<0.1
NOTE: All regressors at their mean value

Latent Class Linear Regression Results

The LCM predicted 2 Classes of consumers. Table 8 shows the predicted probabilities of households belonging to each of the classes. The probability of a household in the sample belonging to Class I is 72.1% while belonging to Class II is 27.9%.

Table 8. Estimated Class Probabilities

Class	Probability
Class I	0.721
Class II	0.279

Note: * p<0.1, ** p<0.05, *** p<0.01

Table 9 shows class membership, which is determined through age and employment. By examining the results in Table 9 further, we refer to Class I households as 'Conservative' households and Class II households as 'Progressive' households. This is because the Conservative class of households show some common characteristics of fish consumption among Ghanaian households, especially in rural and peri-urban areas. According to Heinbuch (1994), consumers in rural households choose fish because it is cheap while consumers in the urban areas consume fish for health and nutritional reasons. For Progressive households, employment is a stronger determinant (12.2%) for class membership while age is the major determinant for Conservative households (96.2%).

Demand for fish is price inelastic among Conservatives but relatively more elastic compared to Progressive households, which is approximately unitary elastic. When the price of fish increases by 1%, the consumption of fish decreases by 0.92% among Conservative households and approximately 1.00% among Progressives (Table 9). For Conservative households, fish is complementary to poultry but a substitute for red meat. Progressive households also consider poultry as a complement to fish but red meat and pork as substitutes. Potential reasons for the substitutability of fish over other animal proteins are economical, health and nutrition related factors and taste (Heinbuch, 1994). Price is a major concern for consumers in the rural and peri-urban areas, who tend to be more Conservatives while taste, dietary diversity, health and nutrition concerns pertain to urban consumers, who tend to be more Progressive. The literature has largely reported the health benefits of eating fish relative to other types of meats (Lajous et al. 2012; Wurtz et al. 2016, Sui, et al. 2016), which might be some of the reasons for the observed shift from red meat to fish in households. Goat meat, beef and pork (in the form of pig feet) are quite popular among Ghanaian households, but are consumed in small quantities in rural areas. Pork and poultry are popular in households in urban areas (Essuman, 1990).

Table 9. Estimated Parameters of the Latent Class Linear Model for the 2 Classes

Variables	Conservatives	BSE	Progressives	BSE
Fish price	-0.922**	0.037	-0.998***	0.000
Poultry price	-0.053***	0.024	-0.001***	0.000
Red meat price	0.107**	0.023	0.001***	0.000
Pork price	-0.053	0.033	0.001***	0.000
Akan	0.118**	0.054	0.007***	0.001
Ewe	0.067	0.071	0.013***	0.001
Ga	-0.110	0.086	0.002	0.001
Guan	-0.035	0.077	-0.029***	0.002
Dagomba	-0.168**	0.084	0.003***	0.001
Islamic	-0.060	0.061	0.000	0.001
Christian	0.039	0.046	0.001*	0.000
Coastal	-0.049	0.055	0.001*	0.000
Forest	0.173***	0.041	-0.003***	0.000
Savannah	0.182***	0.058	0.003***	0.001
Education	-0.016***	0.005	0.000**	0.000
Monthly income	0.126***	0.021	-0.001***	0.000
Married	-0.040	0.031	0.002***	0.000
Male	0.010	0.030	-0.004***	0.001
1st quarter (Q1)	-0.129***	0.042	-0.006***	0.000
2nd quarter (Q3)	0.026	0.051	-0.001*	0.000
3rd quarter (Q4)	0.137***	0.042	0.003***	0.001
Constant	2.959***	0.170	0.008***	0.002
Fixed Parameters				
Constant	-0.509***			
Age	0.038***			
Employed	-0.122**			

Note: * represents p<0.1, ** p<0.05, *** p<0.01, BSE = Bootstrapped standard errors with 100 replications

The FAO (2002b) reported that human diets have evolved significantly in developing countries largely influenced by rapid urbanization. Changes in population and income levels are resulting in dietary diversity in the urban areas of Accra, Kumasi, Sekondi-Takoradi and Cape Coast in Ghana (Osei-Asare and Eghan, 2014). Such lifestyle aligns more with Progressive households.

Fish consumption among Conservative households increases by 12% if they belong to the Akan ethnic group, and by 17% and 18% respectively if located in the forest and savannah areas (Table 9). Fish consumption by Progressive households increase by 0.7% when they are affiliated with the Akan ethnic group and by 1.3% and 0.3% respectively when they belong to the Ewe and Dagomba ethnic groups. Consumption of fish also increases by 0.1% and 0.3% respectively if a Progressive household is in the coastal and savannah areas and by 0.1% if they identify as Christians. The relative impact of the ethnic and location variables is generally stronger with Conservatives than the Progressives (Table 9).

The fish elasticities obtained for Conservatives and Progressives are typical for households located in the forest, coastal and savannah areas. Forest and savannah dwellers tend to consume less fish compared to coastal dwellers due to availability because of proximity to landing sites. This could also explain the relatively less-price elasticity of fish to Conservative households and the substitutability of red meat because of readily available red meat in the forest and savannah regions. The unitary price elasticity of fish among Progressives could indicate the availability of fish because of proximity as well as health and nutrition concerns.

The impact of education is mixed. An additional year in school decreases the fish consumption by 1.6% among Conservative households (Table 9). Education seems to have very minimal effect on fish consumption in Progressive households. Progressive households may be more conscious about the health benefits of animal proteins in general, therefore their consumption of fish may not be driven by education level but their lifestyle, taste and preferences. Conservative households, on the other hand, may be driven by economic factors.

Household income is one of the major variables that significantly impacts fish expenditures. With an increase by 1%, the demand for fish increases by 0.13% among Conservative households, but decreases by 0.001% among Progressive households (Table 9). Because the expenditure on fish is increasing when income is increasing, we can assume that fish is a normal good to Conservative households but an inferior good to Progressive households. The results appear to make economic sense because according to the Engle's Law, high-income households tend to lean more towards dietary diversity, taste and nutrition rather than quantity (Jensen and Miller, 2011) while meeting their daily caloric requirements. Similar results are reported in the literature. For example, Amao (2006) found that for urban households, fish is an inferior good since income elasticity is negative. Tambi (2001) also reports result for high-income households in Cameroon and observed that they reduced their fish consumption with an increase in income. However, contrary results were reported by Anyanwu (2014) who analyzed the socioeconomic drivers of fish consumption among households in Nigeria and reported that fish is a normal good in high income households.

Marital status and gender of household head significantly impact fish consumption by Progressive households and not Conservative households, especially when the household head is married and a female (Table 9). However, male-headed households have been reported to influence fish consumption in Nigeria (Anyanwu, 2014). In Ghana, the woman is mostly the one that prepares meals in typical households and may decide what the household consumes for the day. In urban regions, gender roles are becoming less rigid because food-away from home is more available, and there is no clear indication of who makes the decision of what to eat (Lee and Tan, 2006, Ham and Yang, 1998).

However, the result obtained here corroborates studies on the effect of gender and households on food consumption, where households with female heads tend to have positive consumption of animal source protein in general while male heads tend to decrease the consumption of such foods (Plataroti, 2016).

Processed Fish Expenditure by Location and Ethnicity

The hypothesis that location and ethnic affiliation have no effect on fish consumption was tested using the Wald test of linear restrictions. The chi-squared value for ethnic affiliation is 10.29 and significant at the 1% level. The chi-squared value for geographical location is 11.31 and significant at the 1% level. This implies the variables are very important and that producers can take into consideration geographical location and ethnic affiliations in their seafood marketing approach to consumers.

A further analysis into the diverse types of processed fish consumed by location indicates that on average, smoked fish is the most consumed processed fish by all households (Figure 1). Households in the forest and coastal areas consume more smoked fish compared to the national average. Households in the savannah areas consume more dried fish while households in GAMA consume more fried fish. Fresh, frozen fish and crustaceans are consumed more in GAMA households. This is because they are relatively more expensive and households located in GAMA have higher purchasing power so they can afford and their tastes vary because of changes in lifestyle. Crustaceans on the average are the least consumed followed by salted and canned fish. Salted fish (*Koobi*) is a delicacy among households located in the forest and coastal regions. Figure 1 aligns with the 1978/1999 household survey as well as the 2008 fifth round of the GLSS indicating that fish expenditure has generally increased as a percentage of the overall Ghanaian households' food budget. The inelastic nature of fish price among Conservatives is also substantiated in Figure 1 with the forest region consuming the most fish. Even though Coastal and GAMA households also consume smoked fish, they consume the greatest amount of fresh fish. This supports the argument of health conscious consumers, normally found in urban areas preferring fresh foods to processed foods.

Expenditure on processed fish by ethnic group is presented in Figure 2. The Guans, Ewes, Akan, Ga and other ethnic groups consume more smoked fish while the Dagomba ethnic group consume more dried fish. Households affiliated with the Ga ethnic group also consume more fried fish than the national average. The Guan, Ga and Ewe ethnic groups have relatively higher consumption because of their proximity to landing sites (e.g. Tema port, Chorkor and the Volta Lake). This also explains their relatively higher consumption of fresh and frozen fish. The Dagomba ethnic group consume more dried fish because they are further away from fish landing sites and dried fish stores better than smoked fish, particularly for households.

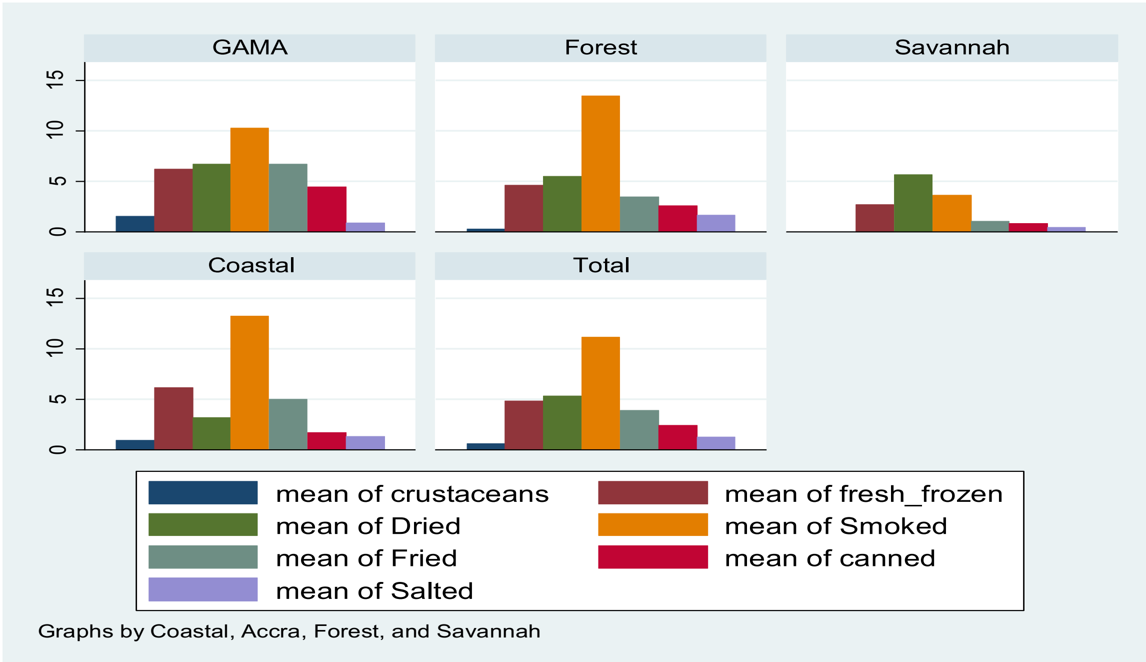

Figure 1. Expenditure for Processed of Fish by Location

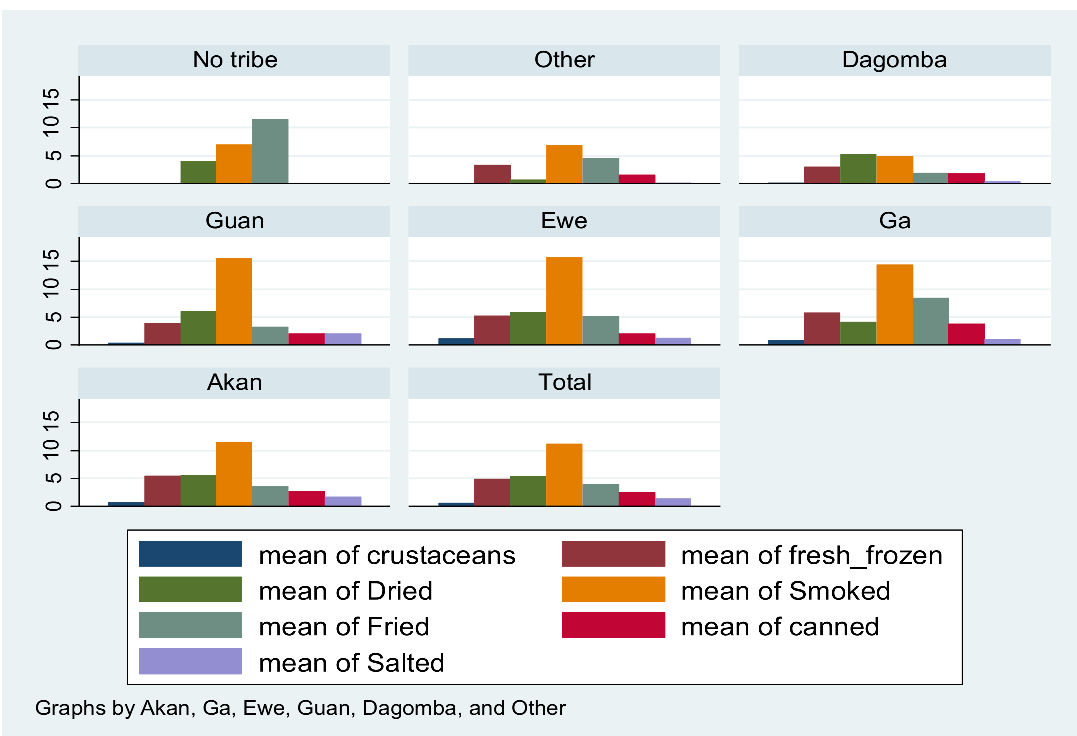

Figure 2. Expenditure of Processed Fish by Ethnicity

Policy Recommendations for Ghana

1) The findings suggest the promotion of fish farming in the three Northern regions, preferably aquaculture in water reservoirs using cages. The Northern regions are the least developed in the country, and cage fish farming requires low capital cost compared to land based fish farms. With

efficient stocking density, good returns on investment can be realized, and it requires very little labor and less maintenance. These advantages make cage culture quite suitable for low-income households. The existing irrigation schemes in the Northern and Upper East regions (Bontanga, Golinga, Ligba, Vea and Tono) for rice and vegetable production will be good sources of water for the cage fish farms.

2) Women should be encouraged to engage in more than processing of fish and getting involved in fish production as well. The simulation results have positive implications for increasing household food security with women as head of household. It is also recommended that further studies be done using a repeated cross section approach to assess the impact fish farming on household food security over a period.
3) Regarding seafood marketing, it is recommended that local fish producers in Ghana be educated on the importance of geographical location and ethnicity on fish demand. Fish producers can take advantage of lifestyles and belief systems to improve marketing of seafood in Ghana by adopting consumer targeting, market segmentation, and positioning strategies in marketing their fish.

RESULTS AND DISCUSSION - TANZANIA

Households in rural areas of Tanzania face barriers that households in urban areas do not face when it comes to access to seafood and food in general. There are differences in access to seafood in the three locations examined, i.e., rural, urban and peri-urban. The results from the two regression using FAC and FAI over location are presented in Tables 10 and 11 respectively. The variables of interests here are the household's expenditure on electricity, communication and transportation.

Regression Results with FAC as The Dependent Variable

From Table 10, the three variables of interest are positive and significant at the 1% level in certain locations. For households in rural locations, increased expenditure on communications, i.e., cell phone airtime implies increased communication between the household and other members of the community, both far and near. This may also imply an increase in the chances of locating the cheapest fish in the situation of price hikes or in the search for variety. As noted by Lashgarara, 2012; and Van Crowder and Fortier (2000), the more access the household has to ICT and information services, the higher the chances of reducing the food insecurity status if the household, which includes accessibility to seafood.

For peri-urban households, an increase in access to electricity increases accessibility by 0.05% with a 1% increase but decreases accessibility to fish by 0.01% with a 1% increase in transportation costs (Table 10). Electricity is a measure of technological access so if households are increasing expenditure on their electricity consumption, they might also have storage and preservation appliances like refrigerators and electric cookers, which increases their access to perishable foods like fresh fish. Peri-urban areas are located on the outskirts of urban areas. An increase in the number of outlets visited will typically mean an increase in transportation costs for household living outside the commercial district or urban areas. Households living in peri-urban areas particularly around Dar es Salaam will benefit from the Dar Rapid Transit (DART), which has been successful since its inception in 2016.

Table 10. Determinants of Household Accessibility to Seafood by Location using FAC

Variables	Rural	Urban	Peri-Urban
Electricity	-0.236 (0.212)	-0.079 (0.054)	0.054***(0.008)
Communication	0.089***(0.027)	0.004 (0.031)	0.008 (0.007)
Transportation	0.019 (0.021)	0.021 (0.006)	-0.014***(0.004)
Price of seafood	-0.581***(0.096)	-0.014 (0.073)	0.024 (0.017)
Age	-0.010** (0.003)	0.006* (0.003)	-0.001 (0.001)
Household size	-0.017* (0.009)	-0.005 (0.015)	0.006** (0.002)
Female	0.184 (0.082)	-0.020 (0.076)	0.011 (0.016)
Married	0.175 (0.107)	0.103 (0.087)	0.044** (0.018)
Unpaid employee	0.019 (0.160)	0.049 (0.154)	-0.049 (0.032)
Paid employee	0.048 (0.139)	0.030 (0.130)	0.008 (0.028)
Education	0.010 (0.011)	-0.001 (0.008)	-0.005*** (0.002)
Household income	0.124 (0.022)	0.001 (0.017)	-0.006 (0.002)
Constant	0.946***(0.251)	1.263***(0.212)	1.280*** (0.046)

Note: *** indicates $p < 0.001$, ** $p < 0.05$, and * $p < 0.10$; Robust standard error in parenthesis.

Age, marital status and years of education of the caregiver, household size and market price of fish significantly influence the household's accessibility to seafood. For households in rural areas, access to seafood is reduced with an increase in the price of fish, age of the caregiver and household size (Table 10).

The high dependence of rural households on food consumptions and variants of food prices directly translate into reduced purchasing power and rising rates of food insecurity, which leads to compromises in dietary quality and quantity (FAO, 2009). While access to seafood increases for urban households with an increase in age. An increase in the years of education reduces access to seafood in peri urban households but increases with household size, and when the caregiver is married.

Regression Results with FAI as the Dependent Variable

Table 11 presents results with FAI as dependent variable. The estimated coefficients with FAI as the dependent variable is assumed less biased and more consistent relative to the coefficients in Table 10. The differences observed are that electricity and communication are significant determinants of seafood accessibility in rural and urban households respectively. Correcting for possible influence of the built-in environment of the household's location increases access to seafood for rural households when expenditure on electricity increases (Table 11). This implies availability of electricity enhances the household's access to seafood. An increase in expenditure in communication in rural households also significantly increase their access to seafood. The magnitude of the coefficient for rural households is smaller compared to the coefficient on electricity in Table 10.

Increase in communication expenditure also increases accessibility to seafood in urban households by 0.02%. Access to cell phones increases the connection among the rural populace and between the rural and urban populations (Batchelor et al., 2005). This may imply that there will be increase in the exchange of information in situations such as price surges and scarcity. Chowdhury et al. (2001) asserts that ICTs contributes to a household's food security by reducing the public and private search costs for food. Findings from Lashgarara et al. (2010) indicate that the use of ICTs is improving access to food in rural households and plays a key role in alleviating food insecurity by decreasing the cost to access information. On the contrary, Olaniyi et al. (2016) assessed the correlation between ICT use and food security in Nigeria and found that the use of cell phones and other communication devices did not improve the food security status of households.

Table 11. Determinants of Household Accessibility to Seafood by Location using FAI

Variables	Rural	Urban	Peri-Urban
Electricity	0.062* (0.036)	0.009 (0.010)	0.054***(0.008)
Communication	0.034***(0.008)	0.021**(0.009)	0.008 (0.007)
Transportation	0.034 (0.006)	-0.000 (0.006)	-0.014***(0.004)
Price of seafood	0.011 (0.024)	0.035* (0.019)	0.024 (0.017)
Age	-0.002** (0.001)	0.002** (0.001)	-0.001 (0.001)
Household size	-0.005**(0.002)	-0.001 (0.004)	0.006**(0.002)
Female	-0.032 (0.021)	0.015 (0.023)	0.011 (0.016)
Married	0.030 (0.039)	-0.013 (0.035)	0.044**(0.018)
Unpaid employee	0.041 (0.049)	0.077* (0.044)	-0.049 (0.032)
Paid employee	-0.001 (0.047)	0.045 (0.031)	0.008 (0.028)
Education	-0.006**(0.003)	0.000 (0.003)	-0.005***(0.002)
Household income	-0.008 (0.006)	0.002 (0.005)	-0.006 (0.002)
Constant	1.357***(0.075)	1.016***(0.059)	1.280***(0.046)

Note: *** indicates $p < 0.001$, ** $p < 0.05$, and * $p < 0.10$; Robust standard error in parenthesis.

Increases in transportation cost reduces accessibility to seafood in peri-urban households by 0.014% (Table 11). The location of peri-urban households provides increased access to a variety of food outlets because of their proximity to urban areas and markets. Although they are close to urban areas, transportation costs associated with increased search time increases because of travel distance between different food outlets. The expansion of the DART system will help in reducing the cost of transportation for peri urban households. In Malawi, the impact of transportation on food access showed that an increase in the travel distance increased per capita daily calories consumed by urban household (Tembo and Simtowe, 2009). Osebeyo and Aye (2014) also found a negative and significant effect of cost of transportation on market participation for smallholder farmers in Nigeria. Improving transportation infrastructure or reducing the cost of transportation increases the probability of improving the welfare of producing households and consumers since high cost of transportation is translated into high food prices. The growing per capita income of peri-urban populations, expansion in access to electricity, refrigeration systems for food storage and transportation facilities have enhanced the growth of supermarkets in peri-urban areas.

The price of fish impacts access to fish by 0.04% for peri-urban households. Urban households may have increased access to a diverse number of food outlets, which increases their accessibility. Supermarkets are commonly one-stop shops, which provide what consumers need and are located in urban and peri-urban areas. Most studies use increased food prices and so the coefficient is always negative. The number of food outlets a household can visit is determined by their income level and market prices (Sakyi, 2012).

Aside the variables of interest, the age, marital status, employment status and the years of education of the caregiver, and household size also significantly influence the household's accessibility to seafood. For rural households, an increase in age is consistent in reducing access to seafood in the two regressions (Tables 10 and 11). Similarly, an increase in the years of education reduces accessibility to seafood for peri-urban households.

Comparison of FAC and FAI Estimates

The differences between estimates from the FAC and FAI models were tested using the chi-squared test. From Table 12, expenditure on communication shows no statistical difference between estimates, but expenditures on transportation and electricity are significantly different at the 1% level. The differences in transportation shows the differences between the estimates because of the differences in the dependent variables. These results show that the FAI is a different measure for access to seafood, though we cannot explicitly say whether the bias is upwards or downwards.

Table 12. Testing Differences in Coefficients between Access Count and FAI Estimates

Variable	Chi sqr. value	P > chi_2
Electricity	51.29	0.000
Communication	2.94	0.229
Transportation	13.36	0.001

Policy Recommendations for Tanzania

1) Studies in the literature on the impact of infrastructure development on the agricultural sector has focused on productivity. However, the outcome of this study also highlights the importance of improving infrastructure in Tanzania, particularly electricity, communication and transportation to improve access to seafood. The DART is a good system that helps to reduce traffic congestion and increase access to seafood and food in general in Tanzania. Continued investments in infrastructure in Tanzania will go a long way to improve seafood accessibility and consequently food security.
2) Access to seafood measures that accounts for the heterogeneous environment may be a better measure than just the access count measure.

CONCLUSIONS

Fish farming households are more food secure relative to non-fish farming households using the Food Consumption Score as a measure of food security. The decision to adopt fish farming is influenced by the wealth of the household, the agro-ecological zone, residing in a peri-urban area, the household size and per capita income of the household. Female household heads and women in general located particularly in the savannah zone (Northern Ghana) will benefit from adopting fish farming particularly cage farming. There is an associated prospect of declining food insecurity.

The per capita fish consumption in Ghana is one of the highest in Sub Saharan Africa. It is a cheap source of protein and common among low-income and subsistence households. Aside price and income being a determining factor in the demand for fish among households, ethnicity and geographical location have been identified as demand determinants among Ghanaian households. Producers can improve consumer targeting and profits by having knowledge of consumers' ethnicity and location.

Tanzania's fish industry is one of the largest in Sub Saharan Africa in terms of production quantities but per capita consumption is 7.7 kg, lower than the global average (19kg). Non-existent and inefficient infrastructure are known to hinder access and availability to fish locally. Increasing access of the household to electricity and communication networks is positively correlated with improvements in seafood access to fish. In measuring accessibility to seafood, it is more accurate to use a more comprehensive measure, which accounts for the environment that the household is located and the type of food outlets available in their location.

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OUTREACH TO INCREASE EFFICIENCY OF AQUACULTURE IN NEPAL

Human Nutrition and Human Health Impacts of Aquaculture/Activity/16HHI03UM

Dilip K. Jha[1], Narayan P. Pandit[1], Nabin B. Khanal[1], Rahul Ranjan[1], Ishori Singh Mahato[1], Madhav K. Shrestha[1], James S. Diana[2], and Hillary Egna[3]

[1]*Agriculture and Forestry University, Nepal*
[2]*University of Michigan, USA*
[3]*Oregon State University, USA*

ABSTRACT

Establishing school ponds and a curriculum for school-age children and women's groups should be an effective approach to create awareness and to educate rural communities about the nutritional value of fish and methods of aquaculture. Two ponds of 75-105 m2 in size were established, one each in two public schools of Chitwan and Nawalparasi districts in Nepal. A school curriculum was also developed to demonstrate methods of aquaculture and educate adolescent students on nutritional value of fish. This technology was also disseminated to women's groups to expand understanding of the value of fish production and consumption for their families.

Carps and Nile tilapia fry were provided to each school from nearby private fish hatcheries and were stocked in each pond at 10,000 carps/ha. In addition to carps, 3,000 Nile tilapia/ha were stocked also. The materials necessary to maintain ponds, including feed and fertilizer, were also provided to each school. Fish were cultured for one production cycle with the participation of high-school-age students. The ponds were maintained in good condition and net yield averaged 4.3 t·ha-1·yr-1, comparable to commercial yields. A course of study was developed for teacher and student education on fish culture. About 40 students of grade 8, 9, and 10 and 3 teachers were selected from each school to receive training on fish culture. Training of teachers and students included fish pond development, managing pond depth, pond preparation, species choice, water color, fertilizing, feeding, grow-out, and harvesting of fish, as well as nutrition education, including fish preparation and eating. Twelve sets of training were conducted in all. Tests indicated that students dramatically improved in knowledge of aquaculture and nutrition after taking this course with median scores increasing from about 60 to 80% in the schools. In addition to training of students, informal education activities were also carried out for women's groups, which included forming two women's fish farming groups in the school community for each district. A training workshop was organized for each women's group on the role of household aquaculture in family nutrition and income. Also, a survey was carried out at four locations to assess the status of fish farmers, their culture practices, and impact of fish farming on economic, nutritional, and social aspects. Results indicated that there was a positive impact of the project on aquaculture practices.

INTRODUCTION

Much of effort to integrate aquaculture and nutrition globally has focused on households. Research is critical in determining best practices and possible variations in aquaculture systems throughout the world and how they might adapt to local culture and conditions (Diana, 2012). However, research alone cannot be effective in changing paradigms in aquaculture communities. Outreach of research results and social interactions to advise local communities are also important in changing aquaculture systems to become more sustainable and more profitable (Diana et al., 2013). Such outreach can target key groups to begin education, with the ultimate goal of local practitioners helping each other improve their aquaculture systems. For aquaculture, direct outreach by government or non-

government organizations is one effective tool, but organic spread of knowledge from practitioner to practitioner is at least equally effective (Tain and Diana, 2007).
Nepal has diverse agro-climatic and socio-economic characteristics, but suffers from limited communication and transportation networks. Most Nepalese live in rural areas at subsistence or near subsistence levels. Most of the protein consumed by the rural population comes from cereal grains. Cereal proteins are generally deficient in one or more essential amino acids and are not complete sources of protein unless taken with other protein sources. An additional concern is that people have a habit of consuming only one cereal grain at a time in Nepal. People in the Terai eat more rice, while those in the hills consume more corn. This tends to make their diets unbalanced in nutritional content. However, this diet may be made nutritionally superior by supplementing it with fish.

Schools remain the center for learning in a community. Having ponds in schools can produce a practical, hands-on message to the local population that fish are an important constituent to boost nutrition, and hence, residents became encouraged to build fish ponds of their own. This also helps build the capacity of teachers who could spread knowledge on the importance of fish in nutrition to parents during teacher-parent interactions, as well as educating students and adults on issues of environmental sustainability and nutrition. We developed a project using school ponds and education on the nutritional value and methods of aquaculture to help young people understand the value of fish production and consumption for their families (Jha et al., 2016). While many Nepalese attend school, most have only a primary school education, and about 68% of women are illiterate. Therefore, training must consider these limitations, while still providing for information exchange (Kloeblen, 2011). The construction and operation of these ponds was a very exciting event for the school communities. Often, a number of adults attended events such as stocking and harvesting, as well as visits during our training exercises.

Women play an integral role in the aquaculture and fisheries sectors all over the world. Although women’s roles and responsibilities are changing in some countries, there are constraints that limit female participation in aquaculture (Egna et. al., 2012). A few such constraints women face in aquaculture and fisheries are time availability and allocation, land ownership and access to water, credit, training, and labor. Lack of training opportunities can trap women in vulnerable and poorly paid positions with no prospects of advancement (FAO, 1998).

Since various outreach activities have been a major component of our work in Nepal, we also believe it is time to evaluate the relative success of these activities. For this component, we focused on locations where people have received training or on-farm experiments to improve local fish culture. We conducted a survey to determine how many of our innovations have been included in the common culture practices of local people. We realize farmers may not easily implement changes in practice completely, but may make modifications to their production systems based on how they perceive the practice to improve their yield or profit.

There is a global concern that nutritious food must be supplied to women, as well as their children during the first 1,000 days of life. Fish provides valuable nutrients to the world’s population, including high-quality proteins (about 6% of world protein supply in 2002); balanced amino acids; vitamins A, D, and B12; iodine and selenium; and long-chain omega-3 polyunsaturated fatty acids. Fish bones, when eaten, are also an excellent source of calcium, phosphorus, and fluorides. Fishes are acceptable for most of the ethnic groups in Nepal and have formed one of the very important parts of human diet since the time immemorial. The fact that fishes are readily available sources of generous amounts of complete protein and of a great variety of vitamins and minerals make them a valuable food for women during pregnancy and lactation, for children during their periods of growth and maturation and for our growing population of senior citizens (Jha, 2018). At least a three-fold increase in animal protein supply is required for optimum health of many rural people. Nepal should promote small-scale aquaculture by setting immediate and long-term objectives. The immediate need is to increase awareness among rural communities of the potential for backyard fish farming, while in the long term, commercial aquaculture should be encouraged (Bhujel et al., 2008). Also, fish contribution to household food and nutrition security depends upon availability, access, and cultural and personal preferences (Beveridge et. al., 2013).

Fish has been considered “living cash” and a pond a “saving bank” because fish can usually be harvested throughout the year when needs arise (Bhujel et al., 2008, Shrestha et al., 2012). Based on the previous result of our school pond program, educational efforts were focused on to create more awareness on different cultural practices for better fish production and consumption.

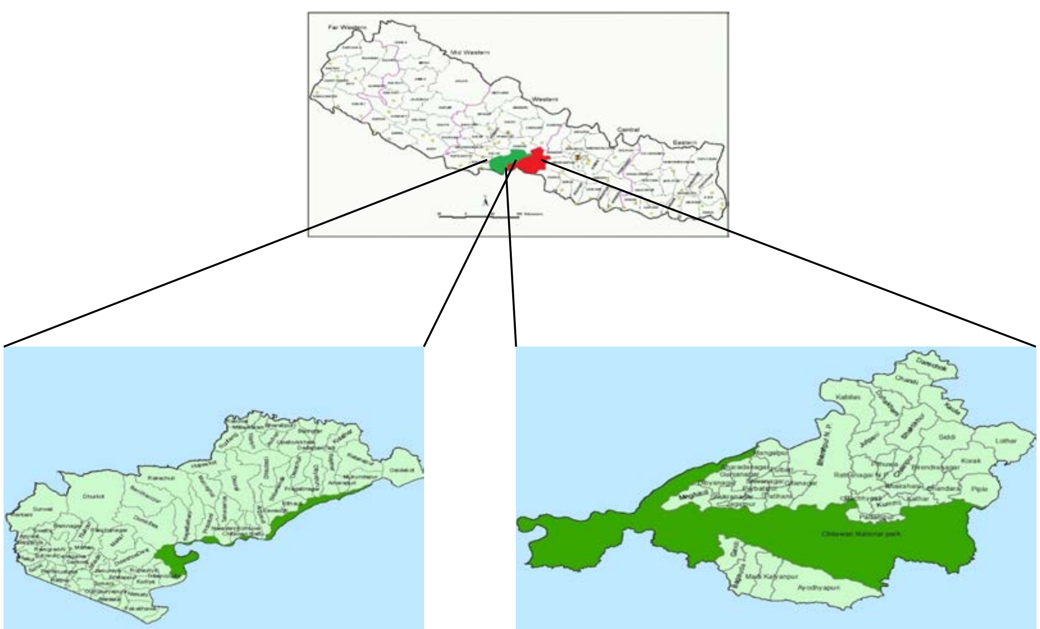
OBJECTIVES

The overall goal of this project was to educate rural communities on aquaculture practices and nutritional value of fish through establishing pond in the school and training students, teachers and women. The specific objectives were:

1. To expand outreach on school ponds in villages and methods of aquaculture in general to the communities near our target schools.
2. To conduct surveys to determine recent changes in fish culture practices in rural areas of Nepal and the sources of information that led to these changes.

MATERIALS AND METHODS

Two public schools were selected in Chitwan and Nawalparasi districts of Nepal (Figure 1). Ponds approximately 75-105 m^2 in area were established at each school (Table1). Ponds differed in design due to land available for a pond, location within the school, and soil permeability (Figure 2 and Figure 3).



Nawalparasi district   Chitwan district

Figure 1. Map of Nepal showing the location of Chitwan and Nawalparasi districts

Table 1. Name of schools, location, size, and type of fish ponds in the present study

School Name	Address	Pond Size	Pond Type
Annapurna Secondary School	Parwatipur, Chitwan	10 m x 7 m	Concrete tank
Chandeswori Secondary School	Godar, Nawalparasi	15 m x 7 m	Earthen pond with plastic lining

Figure 2. Fish pond constructed at Chandeswori Secondary School, Godar, Nawalparasi

Figure 3. Fish pond constructed at Annapurna Secondary School, Parwatipur, Chitwan

Carps and tilapia were provided for each school from nearby private fish hatcheries, and were stocked in each pond at 10,000 and 3,000 fish/ha, respectively. The carp species included silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), stocked at a combined density of 10,000 fish/ha. In addition to carps, monosex tilapia (*Oreochromis niloticus*) were added to each pond at 3,000 fish/ha. Stocking ratio of different carps (%) is given in Table 2. The materials necessary to maintain ponds, including feed and fertilizer, were provided to each school. One cycle of fish culture was demonstrated with the participation of school teachers and students.

Table 2. Stocking ratios of carp at each school

	Carp stocked (% of total)						
School	Silver	Bighead	Common	Rohu	Naini	Grass	Tilapia
Annapurna	14	11	21	11	7	7	21
Chandeswori	21	16	32	16	11	11	31
Species Composition	20%	15%	30%	15%	10%	10%	22%

A course of study was developed to educate teachers and students about fish culture. About 40 students from grades eight, nine, and ten and three teachers were selected from each school to receive regular training on fish culture. School students and teachers received regular training from the principal investigators and other experts about fish nutrition, pond measurement and farming activities, with similar lessons for both groups. Training of teachers and students included fish pond development, managing pond depth, pond preparation, species choice, water color, fertilizing, feeding, grow-out, and harvesting of fish. In addition, we included nutrition education, including fish preparation and eating, in the training. Twelve sets of training were conducted in total. Students and teachers were expected to be responsible for long-term maintenance, sales, and income generated from the school ponds.

We formed two women's fish farming groups in each school community for further training activities. A training workshop was organized by the project team at each school with a women's group. The topic was the role of household aquaculture in family nutrition and income. A linkage was developed so the women's fish farming groups could ultimately work with the teachers and students to ensure long-term sustainability of the school ponds.

Two tests were designed to evaluate the knowledge of students in fish pond production, as well as their knowledge about the benefits of fish nutrition. The tests were administered before and after training given in each school system.

Also, a survey was carried out at four locations in Chitwan and Nawalparasi districts of central Nepal to assess the status of fish farmers, their culture practices, and impact of fish farming on economic, nutritional, and social aspects.

District	Location	Program carried out by AquaFish	Number of farmers surveyed
Chitwan	Khairahani 10	School Pond, CST* polyculture	50
Chitwan	Kharahani 12	Periphyton enhanced aquaculture	50
Nawalparasi	Kawasoti	School pond	50
Nawalparasi	Madhyabindu	Periphyton enhanced aquaculture	50

* CST = Carp, Sahar and Tilapia polyculture

Khairahani 10 in Chitwan district included Kathar and adjacent area and was considered as Chitwan-1 during survey, while Khairahani 12 included Majhui, Kapiya and other adjacent areas and was considered as Chitwan-2. Similarly, Kawasoti in Nawalparasi included several wards of Kawasoti Municipality and was considered as Nawalparasi-1, while Madhyabindu in Nawalparasi included several wards of Madhyabindu and Devchuli Municipality and was considered as Nawalparasi-2 during survey.

RESULTS AND DISCUSSION

Two public schools were selected for establishment of school ponds in Chitwan and Nawalparasi districts of Nepal (Table 1 and Figure 1). Ponds differed in design due to land available for a pond, location within the school, and soil permeability.

Ponds were initially stocked on different dates and harvested after 180 to 210 days (Table 3). Stocking ratios varied among schools due to availability of fry, but all ponds were stocked at 1 carp per m^2 (Table 2), and 0.3 tilapia per m^2. The students proved to be very good at fish culture, with yields in these ponds similar to commercial aquaculture in the region (averaging 4.5 $t \cdot ha^{-1} \cdot yr^{-1}$, compared to 4.9 $t \cdot ha^{-1} \cdot yr^{-1}$ for commercial aquaculture in the country) and overall survival (carps+ tilapia) ranging from 72 to 80%. Due to small sample size (only 2 school ponds), statistics could not be done on these results. However, tilapia showed better survival than carps (Table 4). In addition, feed conversion efficiency was relatively good averaging 1.9 (1.7-2.1). Grow-outs were conducted through both summer and winter months, with water temperatures averaging about 29.2°C and ranging from 18.3 to 32.7°C. Water quality was never an issue during grow out (Table 5).

Table 3. Stocking and harvest data for each school pond

School	Pond Area (m^2)	Total stocked weight (Kg)	Total harvested weight (Kg)	Culture period (Days)
Annapurna	70	0.815	14.01	210
Chandeswori	105	1.240	29.09	180

Table 4. Extrapolated net yield, gross yield, survival, and feed conversion ratio (FCR) for each school pond.

School	Net yield (T ha^{-1} yr^{-1})	Gross yield (T ha^{-1} yr^{-1})	Tilapia survival (%)	Carp survival (%)	Overall survival (%)	FCR
Annapurna	3.3	3.5	95.2	77.3	79.8	2.1
Chandeswori	5.4	5.6	87.1	69.8	72.3	1.7

Table 5. Important water quality parameters measured by the students. Mean±SE (range)

Parameters	Annapurna	Chandeswori
Temperature (°C)	29.9±3.4 (18.3-31.8)	28.6±4.8 (22.4-32.7)
Dissolved Oxygen (mg/L)	3.6±1.1 (3.2-6.8)	4.5±1.2 (2.1-5.8)
pH	7.6 (8.1-8.3)	8.2 (7.3-7.9

Two tests were designed, before and after training to test the knowledge of students in fish pond production, as well as their knowledge about the benefits of fish to human nutrition. For each school, median grades for pre-tests were <40%, increasing to post-test medians near 80% (Tables 6). On the general perception of aquaculture and nutrition, the percentage of students obtained score below 40 was 25.6 % before training, while it decreased to 0 after training. Similarly, the percentage of students obtained score between 40- 60 was 48.2 % before training, while it decreased to 11.5% after training (T-test, $p<0.05$, Table 6).

Table 6. The percentage of students receiving different scores for tests related to overall aquaculture and nutrition taken before and after completion of the school pond class. Mean values with different superscript in the same row of same score range are significantly different (T-test, $p<0.05$).

	Before training				After training			
Score	<40	40-60	60-80	>80	<40	40-60	60-80	>80
Annapurna	40.0	52.0	8.0	0.0	0.0	17.5	32.5	50.0
Chandeswori	11.1	44.4	44.4	0.0	0.0	5.4	45.9	48.6
Mean	25.6±20.4[a]	48.2±5.4[a]	26.2±25.7[b]	0.0±0.0[b]	0.0±0.0[b]	11.5±8.6[b]	39.2±9.5[a]	49.3±1.0[a]

Tests results also indicated that students' initial knowledge about the nutritive value for fish prior was quite poor, with 51% of the students scoring <40 on the test, while it significantly decreased to 0% after training (T-test, $p<0.05$). Similarly, the percentage of students that scored between 40 and 60 was 32.5% before training, while it decreased to 15.5% after training (Table 7).

Table 7. The percentage of students receiving different percentage scores for nutrition related questions taken before and after completion of the school pond class. Mean values with different superscript in the same row of same score range are significantly different (T-tests, $p<0.05$).

	Before training				After training			
Score	<40	40-60	61-80	>80	<40	40-60	60-80	>80
Annapurna	66.0	26.0	6.0	2.0	0.0	17.5	32.5	50.0
Chandeswori	36.1	38.9	25.0	0.0	0.0	13.5	48.6	37.8
Mean	51.1±21.1[a]	32.5±9.1[a]	15.5±13.4[b]	1.0±1.4[b]	0.0±0.0[b]	15.5±2.8[b]	40.6±11.4[a]	43.9±8.6[a]

Students' initial knowledge about farming practices for fish prior was quite poor, with 18% of the students scoring <40% on the test, while only 5% scored <40% after training (T-tests, $p<0.05$). Similarly, the percentage of students obtained scores between 40% and 60% was 26.9% before training, but only 6.5% after training (Table 8).

Table 8. The percentage of students receiving different scores for aquaculture related questions taken before and after completion of the school pond class. Mean values with different superscript in the same row of same score range are significantly different (T-tests, $p<0.05$).

	Before training				After training			
Score	<40	40-60	61-80	>80	<40	40-60	60-80	>80
Annapurna	28.0	26.0	38.0	8.0	10.0	7.5	32.5	50.0
Chandeswori	8.3	27.8	61.1	2.8	0.0	5.4	43.2	51.4
Mean	18.2±13.9[a]	26.9±1.3[a]	49.6±16.3[a]	5.4±3.7[b]	5.0±7.1[b]	6.5±1.5[b]	37.9±7.6[b]	50.7±1.0[a]

It's difficult to attribute many changes in behavior to this program, due to the short time period for training (one year) and variability in consumption by individual households. There was no significant increase in household fish consumption after completion of the course, though it was 30.8kg per year after training and 23.7kg before training (Table 9). Approximately 17% of students lived in households with fish ponds, and one household built a new pond during the training period (Table 9).

Table 9. Details on students trained, students with household ponds, and fish consumption of students (number of times fish were eaten per year) for program students before and after completion of the school pond class. Mean values with different superscript in the same row are significantly different (T-tests, $p<0.05$).

School	Number of Students		Number with a Fish Pond		Fish Consumption (kg)	
	Before Training	After Training	Before Training	After Training	Before Training	After Training
Annapurna	50	40	6	7	22.3±27.8[a]	23.4±16.9[a]
Chandeswori	36	37	7	7	25.6±44.7[a]	38.9±40.3[a]
Mean	43	39	6.5	7	23.7±35.6[a]	30.8±31.2[a]
Total			13	14		

Establishing school ponds and a curriculum for school-age children and women's groups should be an effective approach to educate rural communities about the nutritional value of fish and methods of aquaculture. To create linkages with the community, this program organized two women's fish farming groups in the school communities. Members learned about the importance of household aquaculture for nutrition and income generation. Connecting the local women's groups to the school pond project spread the value of fish production and consumption among households and ensured long-term school pond sustainability.

Survey results indicated that there was a positive impact of the project on aquaculture practices. All aquaculture development programs, including trainings and experiments, were expected to cause some change in culture practices as well change in economic aspects of farmers. Table 10 shows the changes in aquaculture adopted by farmers on getting trainings.

Table 10. Changes in culture practice after training

	Chitwan-1	Chitwan-2	Nawalparasi- 1	Nawalparasi- 2
Change in culture practice				
Y	21	43	41	35
N	29	7	9	15
Type of change				
Feed	13	37	12	8
Fertilizer	4	6	24	11
Chemical	10	7	16	10
Disease	7	6	5	2
Water Quality	2	6	7	10
Stocking density	1	8	1	6
Production	3	10	0	11
Marketing	0	1	0	4
Area expansion				
Y	22	8	8	24
N	28	42	42	26
Change in pond design				
Y	26	6	21	24
N	24	44	29	26
Change in farming system				
Y	49	8	24	29
N	1	42	26	21
Change in feed type				
Y	32	45	14	7
N	18	5	36	43

Many farmers in Chitwan-2 and Nawalparasi-1 responded that they have adopted some changes in their culture practice after receiving some training while the number of farmers adopting change was lower in Chitwan-1 and Nawalparasi-2. Changes adopted by farmers in culture practice included feed management, feeding practice, fertilization, disease inspection, water quality management, stocking density, production and marketing strategy. Many farmers responded that they adopted changes in feeding (Figure 4), fertilization, and chemical use. Some respondents (10 from Chitwan-2 and 11 from Nawalparasi-2) also reported to have higher production after adopting new management practices. The largest number of farmers expanding farm size after training was in Nawalparasi-2, followed by in Chitwan-1. However, considerably higher number of farmers adopted some changes in their pond design after receiving training except in Chitwan-2. Changes adopted in pond design related to dyke slope, dyke width, water depth, water inlet, and outlet structures. The highest number of farmers adopting change in their culture system was in Chitwan-1, followed by Nawalparasi-2, Nawalparasi-1, and Chitwan-2. (Figure 5). Figure 5 shows the number of respondents adopting change in feed type after trainings and involvement in experiments.

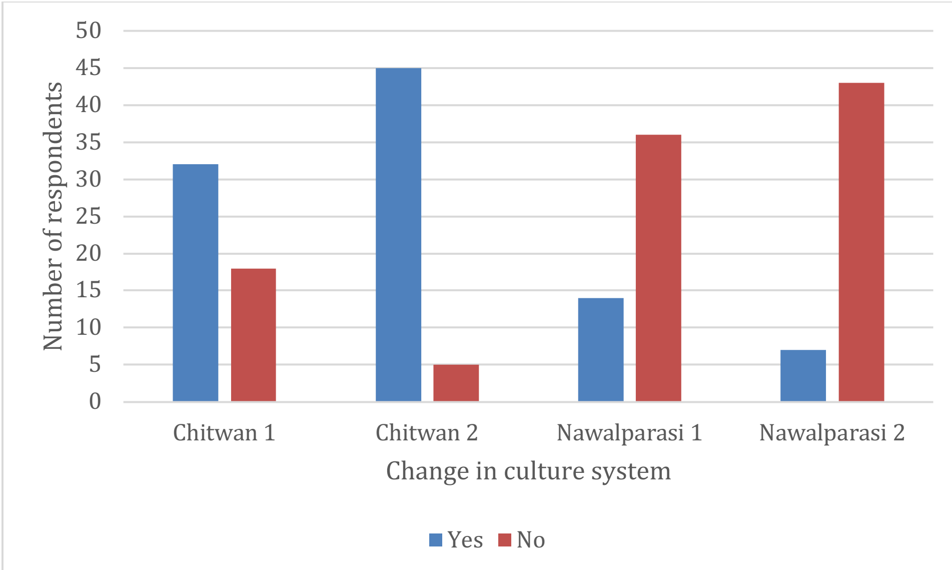


Figure 5. Number of respondents adopting change in feed type after training or involvement in experiments.

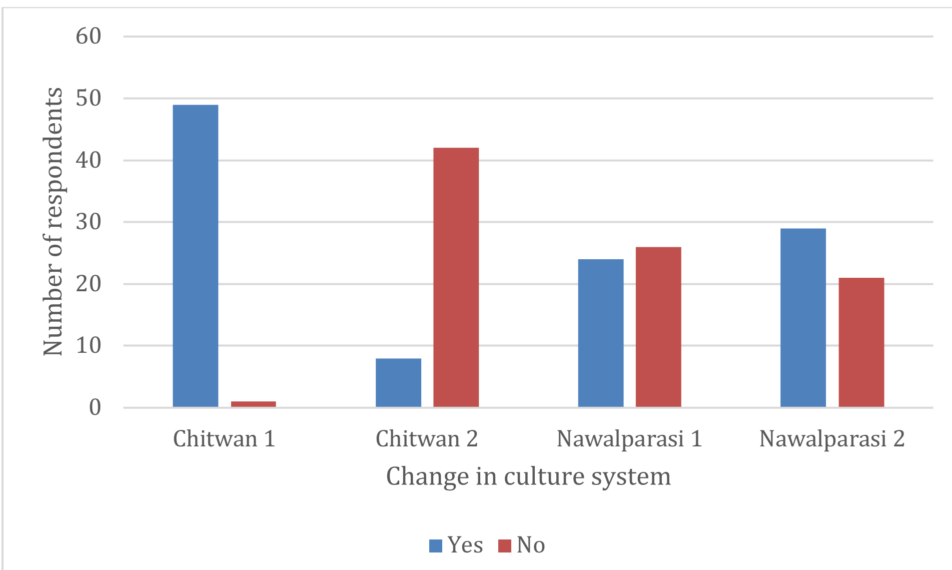


Figure 4. Number of respondents adopting changes in culture system after training and involvement in experiments

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WOMEN IN UGANDA AQUACULTURE: NUTRITION, TRAINING, AND ADVANCEMENT

Human Nutrition and Human Health Impacts of Aquaculture/Activity/16HHI04AU

Gertrude Atukunda[1], Moureen Matuha[1], Namatovu Safina[1], Dorothy Birungi[1], John Walakira[1], Theodora Hyuha[2], Peter Fuuna[2], Gertrude Abalo[3], Claude Boyd[4],and Joseph J. Molnar[4]

[1]National Fisheries Resource Research Institute (NaFIRRI), Uganda
[2]Makerere University, Uganda
[3]Fisheries Training Institute, Uganda
[4]Auburn University, USA

ABSTRACT

The study used discussions and interviews with selected respondents to generate relevant information for training fish farmer groups and other actors involved in aquaculture. The trainings followed data collection so as to make sure that training focused on identified knowledge gaps among target groups. The study focused on selected districts in central and northern regions of Uganda. Purposive sampling was used to identify respondents. Supplementary information was obtained from interviews with members of fish farmer organizations. Findings revealed that women's involvement in the various segments of the value chain performing different roles and activities individually or jointly with men. Factors such as land ownership, decision making over utilization of land, membership to producer and farmer groups, and access to production inputs and extension services influence women's roles in the aquaculture value chain. Participation in farmer groups increases women's opportunities but challenges prevail in the formation, organization and operations of the groups. In addition, specific production and marketing information needs, such as appropriate techniques of feeding fish, processing and marketing fish products to niche markets were noted as directly relevant in enhancing women's role in aquaculture. Stakeholder understanding of the role of fish in child and maternal nutrition is wide and recognized among other key drivers to increasing aquaculture productivity.

INTRODUCTION

This activity addressed a capstone series of events that engaged Uganda AquaFish with institutional partners and the industry to propagate understanding of the nutritional value of a new species and enhance the status and role of women in aquaculture. It built on the previous project by connecting the project of fish farmer cooperatives across the country, women's groups working in aquaculture, and to Nutrition Innovation Lab researchers in Uganda working on nutrition issues so as to amplify and refract the scientific information about fish culture produced by the project.

The work described here endeavors to advance the role women in the provision of fish, an important food item in Ugandan diets. The activities focused on understanding the role of women in aquaculture so as to identify mechanisms for improving household nutrition. Knowledge gaps identified through interactions with women aquaculturalists and fish processors provide basis for right targeting of training content to specific categories of women. We sought to expand the participation of women in production, market development, and use of lungfish and other fish species through training, demonstration, and dialogue among stakeholders. New understanding about how to reproduce and grow this fish will advance farm income and household nutrition.

Poor families in developing countries typically spend between 50 to 70 percent of their income on food (Msangi and Batka 2015). When meat, fish, eggs, fruit and vegetables become too expensive, families often turn to cheaper cereals and grains, which offer fewer nutrients. Widely available,

affordable, and wholesome fish can have profound impacts on human development, particularly in the critical first 1000 days of life (Save the Children 2012). Women tend to cut their food consumption first, and as a crisis deepens, other adults and eventually children cut back. Lungfish are a plentiful source of iron, a critical dietary requirement for children and potentially countering anemia, a significant problem for women.
Lentisco and Lee (2015) identified three main ways in which women access fish as a food item. First is primary access through fishing and financing/owning fishing operations; second is through close personal relationships including family; and third is through the normal purchases in local markets. Fish farming presents a fourth path for women's access to fish. Women producing fish from ponds in Uganda are members of the segment involved in fish-harvesting as primary users; secondary users are those that access fish through kinship or other relationships; and women who buy fish directly from fishers or traders are tertiary users (Lentisco and Lee 2015).

Communication is a fundamental aspect of value chain development and mobile phones have become a central means for advancing these processes. Yet women face continuing barriers to participation. While mobile phone penetration is very high in Africa at almost 80 percent, women in sub-Saharan Africa are on average 23 percent less likely to own a mobile phone (GSMA 2014). One critical obstacle to women's access to mobile phones is affordability: expensive mobiles are reserved for use by men, and women tend to get second-hand phones. Technology is often viewed as a tool for men, so it seems that culture and attitudes toward ownership of productive assets can still be impediments to women's access to technology (GSMA 2014). Trainings and conferences must the address the role of cell phones in women's empowerment.

As aquaculture is often an activity that can be done close to the household, increasing the participation of women can be a strategy for empowerment, but it must be accompanied by secure rights to the resources such as farm space (Lentisco and Lee 2015). For example, lungfish (*Protopterus aethiopicus*) is an emerging culture species in Uganda and there may be opportunities for women to participate in the development of this value chain in a fundamental way (Walakira et al. 2012). The study further shows that lungfish is a popular species marketed in value added form and evident in some women-operated kiosks. In Kampala suburbs and some rural centers, women own the majority of these kiosks, selling fried lungfish chunks and boiled lungfish soup during the evening. report that a small number of restaurants have lungfish on their menu, preparing fresh, smoked and fried fish meals. Some restaurants in Kabusu and Owino centers (Kampala district) specialize in selling fried lungfish pieces.

Following the introduction of aquaculture in Uganda in the late 1940s, aquaculture has been dominated by rearing of fish in earthen ponds operated by households. The practice is regarded a man's activity in accordance with the socio-cultural norms embedded in the patriarchal system that ascribes ownership of household assets such as land to males. Dynamics surrounding ownership of land at household level in turn determine the roles women play in agricultural activities including aquaculture. Similar observations about gender disparity in ownership of land and fish ponds were noted in an earlier study on gender issues in fish farming (Rutaisire et al., 2010). Whereas gender relations influence decisions and actions in undertaking fish farming including utilization of benefits, the level of involvement is not clearly understood to enable identification and analysis of constraints likely to limit achievement of expected benefits (Kruijssen et. al 2018). Despite the limited rights to land ownership, women bear the responsibility of food provisioning for the family.

Women's individual agency is crucial for development as it enhances one's capacity to navigate the psychological, socio-cultural and structural challenges that are faced on a daily basis. It is important that efforts move beyond technical training, although that remains a fundamental, continuing unmet need, to a broader vision of fish farming as a source of nutritional security and income for Uganda families. Women's empowerment is a potential by-product of improved access to inputs and markets, but there is also a need to move to amplify women's roles, agency and voice in this sector. Without direct and active involvement of women the industry will not progress. Some of the gains of empowerment include: women's own income; membership in decision-making bodies; exercise of influence in their communities for aspects that are important for them, such as education for their children and dealing with alcoholism. Lentisco and Lee (2015:21) also cite gains in self-esteem and bargaining power within their households.

OBJECTIVES

1. Train women participating in the value chains of new and established culture species on marketing and nutrition, promoting the understanding of fish as a dietary asset for women and children.
2. Support events among the target populations of fish farmers focusing on women.
3. Develop capacity to access fish production, nutrition, and market information through a series of conferences, workshops, and a national symposium.

METHODS AND MATERIALS

The study combined data collection using discussions and interviews with selected respondents and training during workshops and conferences. The rationale for combining the two approaches was to ensure that training focuses on identified knowledge gaps among target groups. During the training sessions, presentations to fish farmer cooperatives addressed technical needs and issues, as well as specific gender-related concerns in the operation of farmer associations.

The study focused on selected districts in central and northern regions of Uganda (see Figure 1 in Appendices). The two regions differ according a number of characteristics. For example, the central is more densely populated with 176 persons per square meter compared to 65 for the north. Poverty levels are lower (6%) in central compared to 47% in the northern region (UBOS 2016). The central is generally hilly with swamps as main sources of water for fish farming while topography in northern region is mostly low lying with springs as main sources of water.

The respondents were purposively sampled from Bukomansimbi, Kalungu and Lwengo and districts in central region. From the northern region, Alebtong, Gulu, Kole, Lira and Omoro districts were selected. Supplementary information was obtained from interviews with respondents from fish farmer organisations particularly WAFICOS and Women FishNework, based in Kampala. Ogur Fish Farmers' Association in Lira district and Greater Masaka Ssabawali Fish Farmers Cluster based in Masaka were also included in the study. The geographical scope of the study included fish farmers outside Kampala because they are often overlooked by project activities.

Data were analyzed using Atlas.ti computer software where special attention was put on generating results under the themes identified for training activities aimed at advancing the role of women in aquaculture. The themes include aquaculture production, aquaculture marketing, fish and human nutrition, organization of farmer groups/association and women's role in aquaculture.

RESULTS

Development of capacity through training activities and supported events

This activity focused on capacity building of various actors organized in groups and cooperatives, involved in the aquaculture value chain. While most of the groups are comprised of men, women are fairly represented and involved in fish production related activities. The training targeted members of the Walimi Fish Farmers Cooperative Society, the Women Fish Network (both based in Kampala), and selected district based farmers' associations in central and northern Uganda (Table 1).

1. Conducted an edition of the Annual Fish Farming Conference and Trade Show with a focus on women in aquaculture

The Annual Fish Farming Symposium and Trade Show is an activity that brings together various actors in aquaculture. While participation involves men and women, specific groups of women in the aquaculture value chain tend to be underrepresented. The edition of the Annual Fish Farming Conference and Trade Show with a focus on women in aquaculture provided opportunity to reinforce and infuse gender related activities in the ways that women can advance aquaculture in Uganda. The conference was organized by the WomenFish Network in collaboration with NaFIRRI and was attended by 65 women and 35 male participants.

Presentations to fish farmer cooperatives addressed technical needs and issues, as well as specific gender-related concerns in the operation of farmer associations. The importance of ensuring group cohesion was emphasized. Attention was drawn to the social, economic and governance aspects in group operations that often affect groups' attainment of objectives (Stutzman et al, 2017). Characteristics and challenges facing fish farmer groups were pointed out during presentations for purposes of providing lessons to women's groups. Participants

Following discussions carried out in small groups of participants, it was noted that the social aspects underlie effective performance of groups since values such as mutual trust and shared learning are fundamental in ensuring good governance and the attainment of economic gains. Other presentation during the conference highlighted the importance of aquaculture, and the role of women in contributing to income generation, food security and nutrition.

Representatives from the private sector provided useful information about financing opportunities for accessing matching grants to support lawfully registered businesses along the agricultural value chain including the fisheries and aquaculture sub-sectors.

2. Evaluation of women's participation in the aquaculture value chain

Results presented in this section were obtained from discussions and interviews held with women fish farmers, leaders of fish farmers associations and aquaculture extension workers. Results from the discussions elucidate the level of women's involvement in aquaculture. In addition, the discussions helped to identify specific challenges women face in attaining individual and household level benefits from aquaculture.

Results from the discussions were used to map women's participation in the various segments of the value chain as they perform activities individually or jointly with men (Figure 2). While the majority of the women include wives of heads of households and to a lesser extent women heads of households, there are a few employed in the production, processing and marketing segments. Women participate in all pond fish production work though most of the activities are predominantly carried out by men and they include pond site selection and construction, stocking ponds with fingerlings, sampling, sourcing of inputs, harvesting, selling fish, and record keeping. Women are mostly involved in daily routine activities particularly feeding fish, and supervising workers during pond maintenance activities.

The study identified a number of factors that influence women's role in the aquaculture value chain. Some of the factors emanate and manifest at household level while others emerge from outside the household sphere. Among the household level factors is socio-cultural perceptions and practices regarding household resources particularly land. Land is often owned by men who have rights and decision making power on how the land should be utilized including apportioning of the land for different agricultural enterprises. The decision to utilize part of the household land for fish farming lies with the head of the household, who is often a man in the majority of cases. The second factor relates to the decision about whether the purpose of food production is to generating income or to meet home consumption needs. Increasingly, households undertake fish farming as an income generating venture. Men therefore primarily bear the social responsibility of meeting income needs of the household and hence tend to claim ownership of such income generating ventures.

Thirdly, while there are fish farmer groups, membership is often extended to the man because of his ownership status over the fish ponds even when most of the day to day activities are performed by the women. Farmer groups provide opportunities for information sharing, training, access to production inputs and marketing of farm products. While men get opportunities to share benefits of belonging to groups, women remain limited in terms of knowledge and skills needed to enhance their roles. Lastly, limited access to capital negatively influenced the way women operate their agricultural enterprises including aquaculture. During interviews, a female head of household reported having opted to partner with a group of youths to operate her fish ponds on a cost share basis citing lack of capital to repair ponds. The deal provided a win-win situation to both parties since the group of youths lacked land to engage in fish farming but had their own physical labour to renovate the ponds as a way of cutting costs.

Among factors outside the household sphere noted by respondents included extension advice that is often biased towards men who often are ideally regarded as the farmers. In addition, there is limited use of Information and Communication Technologies (ICTs) particularly mobile phones in availing extension advice to fish farmers, a measure that can enable improved access to information by both men and women. The tendency of extension workers to schedule training activities during mid-morning hours that are often not convenient to women was also pointed out. The training schedules coincide with time for preparing lunch hence disadvantages women.

Opportunities and challenges of women's participation in fish farmers' associations

Farmer groups are a form of farmer institutions that were promoted by the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) as a strategy to realize farmer empowerment through effective demand for agricultural advisory services. The groups were formed around priority enterprises agreed upon by members of the farmer group. This strategy was part of the National Agricultural Advisory Services (NAADS) programme introduced following reform of agricultural extension through the National Agricultural Advisory Services Act of 2001. While farmers responded by forming groups at village level, the proposed mode was not favorable to fish farmers because of the isolation by geographical location. Instead, individual fish farmers from a wide geographical area opted to form associations, a strategy deemed as most feasible. Interviews and discussions with members of the associations revealed opportunities and challenges critical to achievement of improved fish production, marketing and nutrition benefits from fish farming. Some of the challenges identified such as weak leadership, inactive members and lack of commitment to towards achievement of stated goal are in agreement with Stutzman et al., (2017). The results provide lessons for appropriate interventions through training of members of the various fish farmers' associations.

Opportunities

1. Big membership (over 50) comprising small and medium scale grow-out and hatchery operators
2. Readily available market in Rwanda and Democratic Republic of Congo (DRC) for large volumes of farmed fish (minimum of 2 tons)
3. Support in form of grants (funds, equipment including training) by government or soft loans by feed companies
4. Processing fish into various products for the local market targeting consumer preferences and nutritional needs of specific categories of consumers such as infants and school children
5. Developing partnerships with national and international organisations/institutions involved in research

6. Developing linkages with similar organisations in the East African Community
7. Gender inclusiveness; women fish farmers are encouraged to join fish farmers' groups even though they are largely dominated by men.

Challenges

1. In-formal membership (no membership and or subscription fees)
2. Lack of institutional recognition/updated registration with relevant authorities such districts or Uganda Cooperative Alliance
3. Weak leadership
4. Weak membership
5. Poor communication between leaders and members and amongst members
6. Poor linkage with relevant local institutions (e.g districts, MAAIF HQs, NARO, NAADS)
7. Lack of operating funds
8. Unclear common goal and demonstrated commitment towards its achievement
9. Inadequate documentation of the operational procedures of the associations
10. Lack of demonstrated visibility of women fish farmers during meetings and other for a of the associations

Aquaculture production and marketing information needs for women fish farmers

While there are a few fish farming households headed by women, majority are households headed by married men. In both types of households, women play significant roles along the aquaculture value chain from production, processing, retailing to consumption. These roles need to be supported through provision of necessary information in order to improve aquaculture productivity. The study noted concerns about how training workshops organized by extension workers target men without recognizing women's roles and information needs.

Analysis of gender division of labor in aquaculture shows women's predominance in pond maintenance work, feeding fish and general supervision activities carried out by employees. Respondents noted lack of information regarding fish feeding, an important activity they carry out on a daily basis. Specifically, respondents needed information on adequate rations and frequency of feeding fish in relation to the culture period. Information to increase fish production was equally expressed in central and northern regions.

Differences in marketing farmed fish existed in central and northern regions due to the varying levels of volumes of fish produced. Fish farmers in the central produced relatively more fish than in the north as evidenced by the type of customers for the fish. While the low volumes of fish produced in the north were easily sold at the pond side, bulk sales were needed in the central. Among the fish farmers in the central region, women headed households produced relatively lower volumes of fish and were sometimes not informed about the opportunity to bulk with other fish farmers and sell to traders from Rwanda and DRC. The women fish farmers needed information about strategies to promptly link with bulk buyers. They also attributed the problem of alienation from information to inactive farmer associations.

For women involved in processing fish, techniques for producing quality products such as fish sausages and fish powder for niche markets in urban areas was the major need. In addition, the main marketing aspects for which they needed information were marketing skills, quality packaging techniques and loans.

Stakeholder understanding of the role of fish in child and maternal nutrition

The study found that understanding of the role of fish in child and maternal nutrition is wide across respondents interviewed. The general perception about the value of eating fish is that it is good for general health of people of all ages and that it boosts appetite among the sick. Respondents in central and northern region cited perceived medicinal attributes of species such as silver fish (*Rastraneobola argenta*) and Haplochromines. The species are perceived to cure measles, kwashiorkor and smallpox among infants. Female respondents however disputed claims that lungfish (*Protopterus aethiopicus*) boosts men's sexual prowess reaffirming that all fish are nutritious. Most of the respondents had knowledge about the health benefits of fish to pregnant women and children under the age of five. The respondents in child bearing age echoed lessons they learn during ante-natal and post natal visits to health centres where importance of good nutrition is emphasized for pregnant and breast feeding women. They also sensitize men about the need to provide good diet to mothers and children. These assertions were reiterated by a leader of a women's savings group in Alebtong district saying:

'…of recent, women are aware of the value of fish because they get information from antenatal visits at the health centers. Also, Village Health Workers trained by the Ministry of Health carry out door to door mobilization of women which has increased antenatal visits. As a group leader, I also preach the same gospel to our members during meetings.'

Consumption of fish was found more frequent in the central than in the northern region. On average, households in the central consumed fish at least once a week compared to about twice a month in the north. This disparity can be attributed to closer proximity of respondents in the central to Lake Victoria. Comparatively, respondents in the north were not close to Lake Kyoga. At the same time though, differences in wealth levels cannot be ruled out as the determinant factor. Households mainly purchased fish from retailers in the markets who obtain it from fish landing sites at the lakes.

Comparison of frequency of consuming animal protein foods showed that fish is most frequently consumed followed by beef and chicken. While fish is more expensive than beef or chicken, social and economic factors influence consumption of fish. In the first instance, respondents reported preference for fish above beef and chicken due to the good taste and perceived health benefits as the main driving factors. In addition, it was explained that fish is readily available in the markets and not sold by weight like meat and therefore provides opportunity to purchase any amount affordable by the buyer. A related factor mentioned was that fish was mostly available in smoked form and in different sizes whereby a buyer can opt for a particular number of whole fish corresponding to number of persons in the household regardless of size of the fish. These results correspond with findings in Kenya where availability, taste, health benefits and price influenced fish consumption and purchasing behavior (Obiero et al., 2014, Githukia et al., 2014).

Regarding consumption of farmed fish, respondents in both regions reported eating fish from their ponds on very rare occasions particularly during harvesting of fish. Occasionally, farmers harvested a few fish from the ponds to avail a special meal for visitors or children who have returned from boarding school.

CONCLUSIONS

The study highlighted issues important to enhancement of women's benefits and participation in aquaculture. The main goals of aquaculture are food security and income generation. Since women are primary providers of food for household members, understanding and enhancement of their role in aquaculture value chain is key. Data collection focused on individuals, households and groups where benefits from aquaculture are realized. Analysis of women's participation in the aquaculture value chain revealed shared roles between men and women implying the need for gender responsive interventions. Farmer groups offer opportunities for improving aquaculture production and marketing but organizational challenges prevail and limit achievement of anticipated benefits.

In particular, understanding of the role of fish in child and maternal nutrition is recognised as a factor that can drive efforts geared at increasing fish production while enhancing the role of women. Training activities conducted for the different farmer groups provided knowledge and skills in ensuring cohesive and active groups that can realize their goals.

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TABLES AND FIGURES:

Table 1. Summary of training activities undertaken

Event	Target Group/category	Number of participants	Focus of training/presentation
Conference onwomen in aquaculture	- Women fish farmers - Women fish processors - Women in academia, policy, research and extension		- Sharing experiences of the different categories of women in aquaculture - Identification of areas for support - Role of Aquaculture in child and maternal nutrition
WAFICOS and WomenFish Network Joint meeting	- Leaders and selected members of WaFICOS - Leaders and selected members of WomenFish Network	26 women 9 men	- Effective Leadership with focus on gender inclusivity - Dealing with group dynamics - Role of women in aquaculture associations - Role of Aquaculture in child and maternal nutrition - Aquaculture fish products [Value addition: fish fingers, deep fried tilapia 20g and below] - Business planning
WAFICOS Annual Symposium	- Fish farmers - Fish processors	37 women 87men	- Role of women in aquaculture associations - Aquaculture and nutrition
Greater Masaka Fish Farmers' Cluster Meeting	- Members of the cluster - Non-members of the cluster - District Fisheries Officers - Representatives of Buganda Kingdom	6 women 25 men	- Role of women in fish farmers' associations/groups - Dealing with group dynamics - Increasing women's participation in leadership roles - Organised fish production and bulk sales to markets in Rwanda and DRC - Partnership with feed companies for feed loans - Raw material for women's fish processing enterprises
Ogur Fish Farmers Association, Lira district	- Members and non-members	6 women 16 men	- Effective Leadership with focus on gender inclusivity - Dealing with group dynamics - Role of women in aquaculture associations - Aquaculture and nutrition - Business skills - Farmer to farmer extension modalities
Kole district fish farmers meeting	- Fish farmers in Kole district	7 women 4 men	- Effective Leadership with focus on gender inclusivity - Dealing with group dynamics - Role of women in aquaculture associations - Aquaculture and nutrition - Farmer to farmer extension modalities
Lwengo, Kalungu and Alebtong district fish farmers meeting	- Fish farmers	22 women 16 men	- Modalities of forming fish farmers association/group - Enhancing women's role in fish farming and farmer groups -

Table 2. Technology developed

Item	**Mechanism (e.g. podcast reports factsheets etc.).**
Conference on Women in Uganda Aquaculture	Fact sheet on women's roles in Uganda aquaculture
One training on women's organizations in development for women farmers and service providers	Leaflet providing practical guidance to women's groups
One training on value chains and marketing in aquaculture for farmers	Leaflet providing practical guidance to women's groups with focus on nutritional value of lungfish
One presentation to fish farmer cooperative members and leaders	Fact sheet for leaders and participants in cooperatives with emphasis on lungfish as a new market item

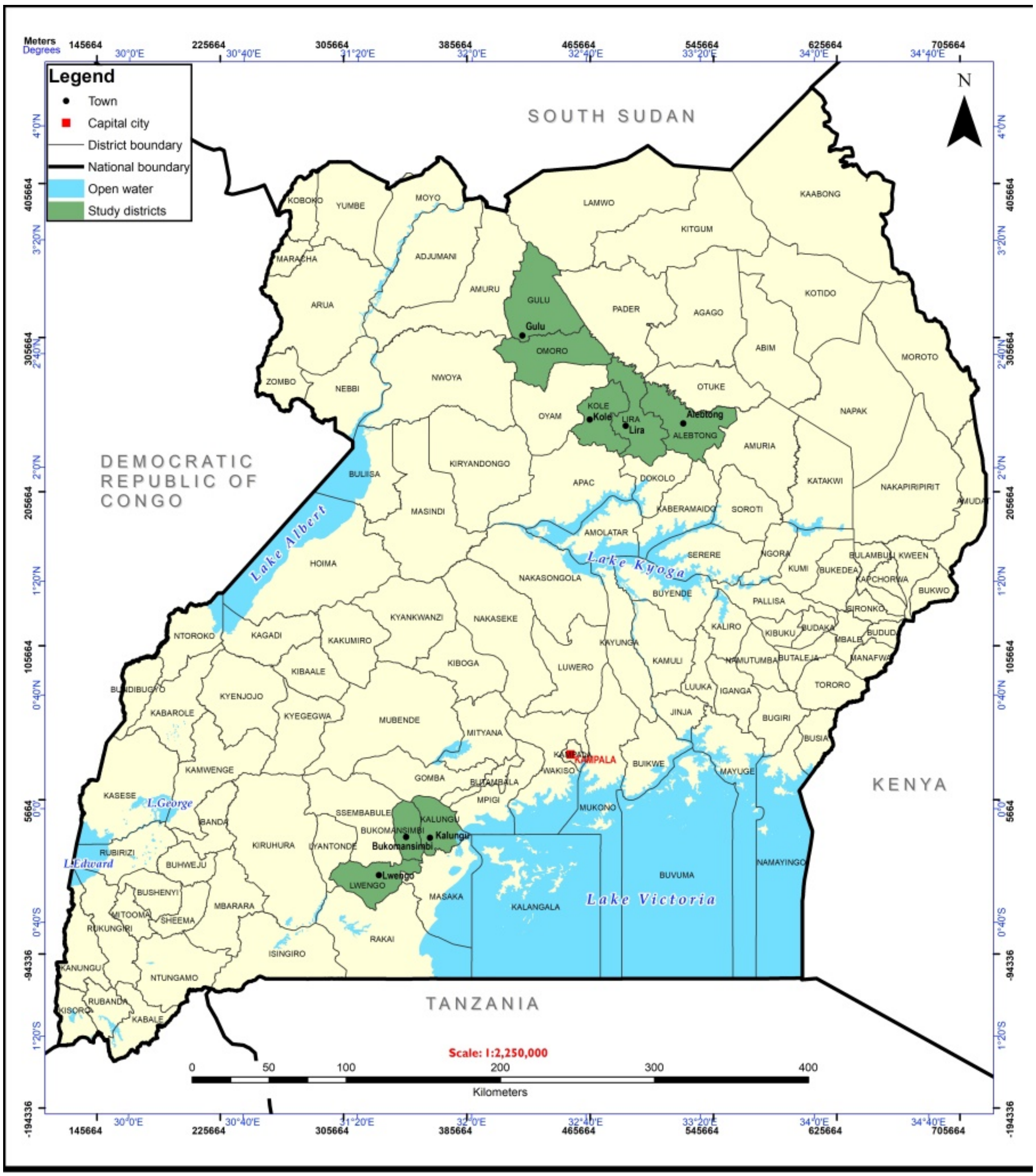

Figure 1. Map of Uganda showing study districts

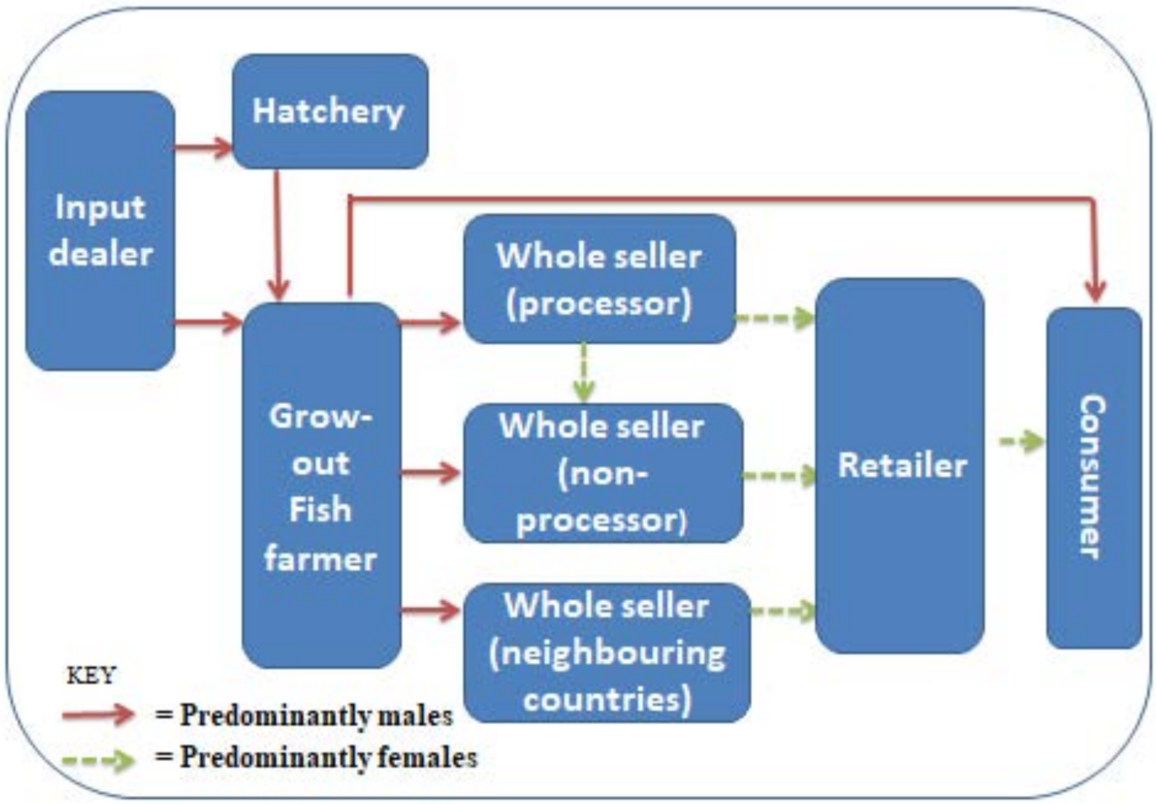

Figure 2. Aquaculture value chain actors by gender (Source: Group Discussions with men and women in central and northern regions of Uganda, 2017).

DETERMINING THE ROLE OF WILD-CAUGHT AND AQUACULTURE-BASED INLAND FISHERIES IN MEETING BURMA'S HUMAN NUTRITIONAL NEEDS

Human Nutrition and Human Health Impacts of Aquaculture/Study/16HHI05MS

So-Jung Youn[1], Jessica Scott[2], Joanna van Asselt[3], Ben Belton[4], William W. Taylor[1], Alexa Lupi[5]

[1]Center for Systems Integration and Sustainability, Department of Fisheries and Wildlife, Michigan State University. 115 Manly Miles Building, 1405 S. Harrison Road, Lansing MI 48823
[2]WorldFish, Myanmar Office. Department of Fisheries, West Gyogone, Bayint Naung Road, Insein Township, Yangon 11181, Burma
[3]International Food Policy Research Institute (IFPRI). 1201 Eye St, NW, Washington, DC 20005
[4]Department of Agricultural, Food, and Resource Economics. 446 West Circle Drive, Room 202. East Lansing, MI 48824
[5]Department of Epidemiology and Biostatistics. 909 Fee Road, Room B601, East Lansing, MI 48824

ABSTRACT

Fish are an important source of food and nutrients in Burma. The contributions fish make to Burmese food security, however, are not well understood. This report analyzed Burmese consumption and nutrient intake from various fish sources (e.g. marine capture, freshwater capture, aquaculture, and dried/processed) in 2005 and 2010. Differences in consumption across zones of Burma, wealth quintile, and urban vs rural were also identified. The data for these analyzes come from the Integrated Household Living Conditions Assessment survey (IHLCA), which was conducted by the United Nations Development Program (UNDP) to assess Burmese household food consumption across a variety of sources, not just fish. Mean fish consumption (kg per capita) in 2010 was less than in 2005 for nearly all categories of species over all regions, locations, and wealth quintiles. It is likely that these results reflect systematic flaws in the sampling and/or data collection process for the 2005 IHLCA since the same pattern is also observed for all other food groups, during a period when incomes were rising and poverty was falling. Moreover, whilst it might be expected that per capita consumption of some capture fisheries species declined during this period due to over-exploitation or habitat degradation, it is difficult to account for such a uniform pattern of falling consumption, especially given that aquaculture is known to have grown significantly during this same period.

Using IHLCA data, national average fish consumption for 2010 was estimated at 20.72 kg/capita. Inland capture fish are particularly important for consumers in rural areas (who are, on average poorer than those in urban areas), while urban consumers and the wealthy are more likely to eat farmed fish. Results also underline the importance of dried, fermented and other preserved fish products. Although often overlooked in assessments of fish consumption, these products account for the single largest category of fish consumed in Burma. Fish, combining all sources (e.g. marine, freshwater, aquaculture) and forms (e.g. fresh, dried, processed) were also a key nutrient source. Fish significantly contributed to per-day intake of protein (21% of recommended intake for women and 17.5% for men), iron (24.4% for women and 55% for men), calcium (24.4% for both women and men), and vitamin B12 (50% for both women and men). Fish contributions for several nutrients (e.g. selenium, vitamin D) could not be determined due to lack of available data for fish species and nutrient compositions.

The findings of this report highlight the importance of fish to Burmese food and nutrition security. By disaggregating nutrient contributions at the species level, we were able to demonstrate the importance of species diversity and the need for nutrition-sensitive approaches and monitoring trends in household fish consumption among the population and geographical groups described.

INTRODUCTION

Fisheries have long played an important role in Burma's rural economy (U Khin 1948; Reeves & Pokrant 1999), and fish a central role in the national diet (Shway Yoe 1966). Fish consumption from inland areas (both wild capture and aquaculture sources) is estimated to provide 30% of total yearly intake of protein (from both animal and plant sources) (FAO 2003). Fish also provide key contributions to the intake of essential fatty acids and micronutrients of high bioavailability such as vitamin B12, iron, iodine, calcium and vitamin A, which can be difficult to obtain through other local food sources and are vital for human growth and development (Aung et al. 2010, Youn et al. 2014). As Burma's most important animal-source food (Belton et al. 2015), fish therefore represents one of the main sources of micronutrients. These nutritional contributions are critically important given that more than 35% of Burmese children show signs of stunted growth, which is linked with chronic malnutrition (World Food Programme 2017).

Understanding the contributions of fish and fisheries to food and nutrition security in Burma is thus important if food and nutrition security and human well-being are to be ensured. These contributions remain poorly understood however, as do the ways in which these are changing over time. This is partly because Burma's half century of political isolation prior to 2011 resulted in an acute lack of data on all sectors of the economy, including agriculture and fisheries (Haggblade et al. 2013), official production statistics for which are considered unreliable (Fujita 2008, BOBLME 2014). Moreover, the production and consumption of fishery resources are often particularly severely misestimated, due to lack of data or political pressure to meet specified government production targets (Bartley et al. 2015, Beard et al. 2011).

Rapid growth, rising incomes and accelerating urbanization linked to Burma's transition to a globally integrated economy post-2011 mean that demand for fish is set to grow: a trend observed throughout Asia as increasingly affluent consumers increase their expenditure on non-staple foods such as fish, meat and fruits (Reardon et al. 2014). At the same time, Burma's capture fisheries resources are reckoned to be heavily exploited. One recent high-profile stock assessment exercise found that the biomass of stocks in the marine fishery stands at 20% of historical levels (IMR, 2014) and inland fisheries are under increasing pressure (MFP 2016a), while aquaculture's potential has yet to be fully realized (MFP 2016b).

Set against this complex and information-poor background, this report estimates the contributions of fish (disaggregated by source – i.e. inland capture, aquaculture, and marine capture; and product type – fresh, processed) to human nutrition in Burma for the first time. These estimates are made using data from a nationally representative household survey, and several nutrient composition databases. Results indicate that fish consumption plays a highly significant role in the Burmese diet, with fresh fish originating from inland fisheries and dried, fermented and other processed fish from marine and inland capture fisheries of particular importance.

METHODS AND MATERIALS

The Integrated Household Living Conditions Assessment survey (IHLCA), conducted in Burma by the United Nations Development Program (UNDP) in 2005 and 2010, is a nationally representative household survey of 18,660 Burmese households conducted by the Ministry of National Planning and Economic Development and the United Nations Development Programme (IHLCA 2011). The purpose of the IHLCA is to provide statistical data for determining living conditions in Burma. To meet this purpose, the survey collected household food consumption data during two rounds in each of the survey years, corresponding to pre- and post-monsoon paddy harvesting season, which captured some seasonal variability in food consumption. Seven-day recall was used to capture the quantity of foods consumed, including the amount sourced through the market, the household's own production, and other source (e.g. gifts).

Fish and fish products were pre-coded as 37 different items in the IHLCA. Fish fall into 3 categories: species specific (e.g. snakehead, Bombay duck), generic but denoting source (e.g. small marine fish), and generic but not denoting source (e.g. fish paste). For fish categories that are generic and do not denote source (e.g. marine, freshwater, or aquaculture), source and composition were inferred, as far as possible, using available information (e.g. it is likely that most fish paste consumed in coastal state is of marine origin). Trade data show that fish imports to Burma are extremely small, at 6029 t/year (FAO 2015a), so import trade should have little impact on the results.

Many of the IHLCA fish categories are species specific, but some items also cover a variety of generic categories of product (e.g. “other small river fish <= 4 inches”, “other dried medium sea fishes”). In these cases, it was necessary to make some assumptions, based on the knowledge of key informants and existing literature, about the most important fish species when conducting nutrient analysis. In particular, the analysis made use of three documents: 1) an assessment of the catch composition from inland fisheries at 14 locations throughout the upper, middle and lower Ayeyarwady and Chindwin River basins (Baran et al. 2017); 2) Unpublished data on the most common most abundant species harvested inshore from three locations in the Gulf of Mottama (Mon State, Eastern Burma), made available by the NGO Network Activities Group; 3) Unpublished data on major species landed from marine fisheries in Rakhine State (Western Burma), made available by the World Conservation Society.

Data from published sources Bogard et al. (2015) and databases (FAO INFOODS INFOODS Global Food Composition Database, Mahidol University ASEAN Food Composition Database, Indian Food Composition Tables 2017, Cost of the diet tool V2 analysis software) were used to obtain estimates of the nutrient composition of the most commonly consumed fish species and fish products. Kilograms of raw whole fish were first adjusted with an edible portion coefficient to exclude parts that are not consumed, e.g. bones. The edible portions were multiplied by the nutrient composition of that species, based on the literature, to estimate nutrient intakes provided by different fish species, and sources of fish (aquaculture, inland capture, etc.) in 2010. Details of the following dietary components were extracted from the literature: Energy, protein, fat, moisture, ash, iron, zinc, calcium, vitamin B12, sodium, iodine, selenium, phosphorous, magnesium, potassium, manganese, copper, vitamin D3, vitamin E, vitamin A, retinol and essential fatty acids. Data was not available for every species/product and nutrient combination. Data on macronutrient composition and iron, zinc, calcium and vitamin B12 content were most complete.

This analysis was conducted at the national and regional level to capture geographical differences in consumption, for rural and urban areas, and by wealth category (as proxied by consumption expenditure per capita) – with households divided into 5 wealth quintiles (quintile 1 representing the poorest 20% of the population, quintile 5 the richest).

IHLCA 2010 is the most recent year of the survey and no recent, comparable datasets exist. Because data for 2005 and 2010 provides a time-series, the intention at the outset of the analysis was to generate estimates of changes in nutrient intakes and production volumes from different fisheries sub-sectors. However, analysis of both data sets indicated that a substantial decline in fish intakes occurred across all species/product groups during this period. Analysis of other non-fish food groups (grains, vegetables, fruits, meat, edible oils) indicated similar declines in consumption. This result is problematic, because the poverty rate in Burma declined significantly over the same period as real incomes rose (World Bank 2015). Declining poverty and rising incomes should be associated with increasing in food consumption, particularly for higher market value non-staple such as fish. Moreover, even in the event that incomes fall, consumers should substitute higher market foods for the staple rice, causing rice consumption to increase. As there is no plausible explanation for why food consumption decreased so sharply across all food groups, the decision was made to exclude

2005 data from nutrient composition analysis. Data from the 2010 IHLCA were considered to be reliable, because reported values for per capita consumption of foods such as fish and rice are within a similar range to those reported in neighboring countries.

Comparison of fish consumption (kg/capita) between 2005 and 2010 is retained for illustrative purposes. Fish species/products, grouped by origin (e.g. aquaculture, inland and marine fisheries; Table 1), were analyzed by region, location (e.g. urban or rural), and wealth quintile. These comparisons were used to identify changes in fisheries production and consumption across Burma between the 2005 – 2010 time-period.

Using the data from the IHLCA 2010, maps were developed to illustrate the contributions of marine and inland capture fisheries and aquaculture to food and nutrition security in Burma. The maps show yearly per-capita consumption of various types of fish by region and identify the relative contributions of aquaculture and inland and marine capture fish to the diet

RESULTS

Fish Consumption in 2005 and 2010

Mean fish consumption (kg per capita) in 2010 was less than in 2005 for nearly all categories of species over all regions, locations, and wealth quintiles. Many of these mean differences in consumption were significantly different (or borderline significantly different) at a 5% level. Exceptions to the decreasing trend (e.g. increases in consumption) were aquaculture species consumption in the East region, all processed fish species consumption in the West region, and processed marine fish species consumption in the West region (Table 2; Table 3; Table 4). However, in cases where there was an increase in mean fish consumption, none of the differences were significant.

Comparing consumption between rural and urban locations for each year, mean fish consumption (kg per capita) in rural locations was generally less than that of urban locations for both 2005 and 2010 (Table 6). Exceptions for both years were freshwater species consumption, all processed fish species consumption, and processed marine fish species consumption, which all had a larger mean fish consumption for rural locations compared to urban locations. However, of all the tests comparing rural and urban mean differences in consumption, only aquaculture species in 2005 was found to be significantly different in rural and urban consumption. Fish consumption also decreased for all wealth quintiles between 2005 and 2010 (Table 5).

As noted above, it is likely that these results reflect systematic flaws in the sampling and/or data collection process for the 2005 round of the survey, given that the same pattern is also observed for all other food groups, during a period when incomes were rising and poverty was falling. Moreover, whilst it might be expected that per capita consumption of some capture fisheries species declined during this period due to over-exploitation or habitat degradation, it is difficult to account for such a uniform pattern of falling consumption, especially given that aquaculture is known to have grown significantly during this period (Belton et al. 2015).

Fish Consumption in 2010

Leaving aside problematic data for 2005, this sub-section summarizes fish consumption (kg/capita/year) patterns for 2010, in terms of geography (area of the country, urban and rural locations) and income (proxied by wealth quintile).

Burma is comprised of six geographical zone: South (Tanintharyi, Mon, Kayin); Lower (Ayeyarwady, Yangon, Bago); West (Rakhine, Chin); Central (Mandalay, Magway); North (Sagaing, Kachin); East (Shan, Kayah). Southern, Lower and Western Burma all have lengthy coastlines, providing access to marine fisheries, and (in some places) rivers and deltaic environments utilized for lowland wet rice cultivation and freshwater capture fisheries. Aquaculture is heavily concentrated in Lower Burma. These three areas, had the highest estimated levels of fish consumption in the country, ranging from 19.4 to 25.1 kg/capita/year.

The estimated contribution of fish to the diet was smaller in the landlocked Central, North and Eastern areas that make up Upper Burma. These areas, where much of the environment is arid, hilly or mountainous, are distant from the main sources of fish production. Consumption of fish was lowest, although still significant, in Eastern Burma, where average estimated consumption stood at 8.5 kg/capita/year.

Focusing on the composition of fish consumption by source (marine and freshwater capture fisheries and aquaculture), and product type (fresh and processed), it is apparent that dried and other processed products account for the largest share of fish consumption nationally (34%). Among fish consumed in fresh form, freshwater capture fisheries account for the largest share (27% of total consumption), with aquaculture and marine capture fisheries contributing 21% and 18% respectively

Processed fish products (mainly dried or fermented) are comprised of a mix of fish and shrimp from marine and inland capture fisheries. Fish from aquaculture are almost always sold in fresh form. Drying and fermenting have traditionally been used to preserve fish which would otherwise spoil quickly, smoothing seasonal gluts and shortages and facilitating trade over long distances in the absence of ice and refrigeration. Although the amount of processed fish in total consumption seems extremely high, it does fall within approximately the same range reported in some areas of the Lower Mekong River Basin with similar aquatic ecologies (Hortle, 2007).

Consumption of aquaculture fish is lowest in West and South Burma, where marine capture fisheries dominate production. Interestingly, although absolute levels of consumption of fish from aquaculture (5.5 kg/capita/year) are highest in Lower Burma, where the vast majority is produced, its contribution to the diet is greatest in Upper Burma (central, northern and eastern areas), where it accounts for 23-32% of total fish consumption. This indicates the existence of considerable domestic trade in farmed fish, allowing the penetration of fresh fish produced in the delta far into Upper Burma. In fact, Upper Burma accounted for 47% of farmed fish consumption, with 43% occurring in Lower Burma, and just 10% in coastal areas (South and West Burma combined). This result highlights the scale and significance of intra-national trade in aquaculture products and its important implications for food and nutrition security in fish deficit areas of the country.

Fish consumption behaviors vary not only among different areas of the country, but between rural and urban zones, and across income groups. Estimated average annual fish consumption per capita is almost the same in both urban and rural areas, at around 21.11 kg/year (urban) and 20.58 kg/year (rural). This pattern is interesting because urbanization (and the higher incomes with which it is normally associated) is usually found to result in overall increases in fish consumption (c.f. Toufique and Belton 2014).

However, aggregate consumption figures mask important differences in the source of fish consumed. Average consumption per capita of freshwater capture fish is 27% higher in rural areas than urban, probably reflecting the nature of the inland fishery, much of which is dispersed and small-scale, making the assembly of sufficiently large quantities to export to urban areas problematic, with the result that much of the catch is consumed locally. Consumption of fish from marine capture and aquaculture is higher in urban areas than in rural (by 25% and 41% respectively). Dried and processed fish is consumed in almost equal quantities in rural and urban areas.

The apparent propensity of urban dwellers to consume farmed fish in greater quantities than their rural counterparts appears to indicate a high degree of substitutability with inland capture fish. This tendency is significant in terms of likely future demand patterns, given that progressively greater urbanization is likely to be a feature of Burma's geography as the country (which is currently in a stage of 'nascent urbanization', with around 30% of the population living in urban areas) integrates further into the global economy, and transitions away from a national economy dominated by the primary sector (World Bank 2015).

Total annual fish consumption per capita among the wealthiest 20% of the population is more than double that among poorest 20% (28.22kg vs. 7.57kg). Disaggregating further, a similar pattern holds for dried/processed fish (10.05 kg vs. 2.51 kg) and freshwater capture fisheries (9.21 kg vs. 2.36 kg). Consumption of fish from marine capture fisheries is more equitably distributed across income groups, with consumers belonging to the wealthiest quintile eating, on average, less than 50% more than consumers in the poorest quintile (4.28 kg vs. 1.94 vs.). Inequality in consumption between rich and poor is greatest for aquaculture fish, average consumption per capita of which is around 6 times higher among members of the wealthiest quintile than among those in the poorest 20% of the population (4.68 kg versus 0.77 kg). From this observation it can be inferred that the income elasticity of demand for aquaculture fish is higher than that for fish from other sources (i.e. an increase in income will result in a proportionately greater increase in expenditure on farmed fish than on fish from other sources).

These findings have main two implications: First, as the economy grows and incomes rise, demand for farmed fish will increase faster than demand for fish from other sources. Second, the relatively unequal consumption of aquaculture fish across income groups, as compared to fish from other sources, reflects the low diversity and rather undifferentiated nature of Burma's fish farm sector (dominated by a single species, Mrigal), equating to limited range of products and prices. Capture fisheries are characterized by much higher diversity of fish species and thus offer "something for everyone", including the poorest consumers. Consequently, there is scope for the development of a more diversified aquaculture sector that caters to a wider range of consumer demand. This possibility has precedents in many other countries in the region, where aquaculture supplies a wide variety of species including those consumed by low-income population groups (Belton et al. 2018).

Nutrient Intakes from Fish

The literature search found no published nutrient composition tables for Burmese fish samples. To complete the nutrient analysis, the literature and data presented represents the best available species and category match from neighboring countries. The data is varied in source and quality of nutrient profile. This can be seen in missing data for certain nutrients across the species and categories and in turn, has reduced the full nutrient profile that can be reported here. Difference in data source aside, there is wide variation in the provision of micronutrients across the range of fish species and groups.

Nutrient composition and intake data were analyzed solely based on the IHLCA 2010 dataset. The data show that fish (combining all sources and forms) were important contributors of some nutrients (Table 8), significantly to per-day intake (HHS and USDA 2015) of protein (21% of recommended intake for women and 17.5% for men), iron (24.4% for women and 55% for men), calcium (24.4% for both women and men), and vitamin B12 (50% for both women and men). A large portion of the calcium and iron contributions come from dried and processed fish products (Table 12), although freshwater fish and fish products are also an important contributor of calcium (Table 10). Despite the large amount of incomplete nutrient data underrepresenting vitamin B12, significance of the contribution of fish can still be seen from the freshwater and farmed fish and products derived from these species (Table 10; Table 11). Additionally, climbing perch, small river fish, and (estimated) Ngapiyae provided the greatest amount of vitamin A, though due to missing data from the whole nutrient composition table, total vitamin A contribution from fish is unable to be reported. Overall, nutrient results should be interpreted cautiously, due to the missing data for many of the micronutrients analyzed.

CONCLUSIONS

Using IHLCA data, national average fish consumption for 2010 was estimated at 20.72 kg/capita. It is striking that it stands at well under half the 50.2 kg/capita fish supply reported in the FAO food balance sheet for 2010 (FAO 2015b). The magnitude of this gap seems to imply that Burma's fish consumption as recorded in the FAO food balance sheet, along with the national fish production figures from which this number is calculated, are significantly inflated. This finding underlines the need for improved monitoring in support of better informed fisheries management and policy decisions.

Results show the continued importance of inland capture fisheries as the main source of fish in most of Burma, while aquaculture is relatively under developed in terms of both levels of production and species diversity, being dominated by Indian major carps. Inland capture fish are particularly important for consumers in rural areas (who are, on average poorer than those in urban), while urban consumers and the wealthy are more likely to eat farmed product) This situation is rather unusual, given that in most other countries in the region aquaculture has already surpassed capture fisheries (in particular inland fisheries) as the main source of fish, with farmed fish now often cheaper than wild (see Belton et al. 2018).

This situation signals two opportunities: First, to implement effective inland fisheries management strategies in order to maintain or enhance levels of productivity from what is still a relatively intact fishery. The value (economic and nutritional) of inland fisheries should also be considered when making decisions regarding developments that may affect the sector (e.g. dam construction for hydropower, water management schemes for agriculture); Second, to provide investments and technical support for the development of a more dynamic and diverse aquaculture sector that is able to supply greater quantities of affordable fish, stimulate rural economic growth and promote livelihood opportunities for farms, workers and businesses in supporting value chains.

Results also underline the importance of dried, fermented and other preserved fish products. Although often overlooked in assessments of fish consumption, these account for the single largest category of fish consumed in Burma. These products are often some of the cheapest animal source foods available and are particularly important in inland areas such as the Dry Zone in Central Burma, were there little local production of fresh fish.

Fish is well recognized as a high-quality animal source protein, rich in essential micronutrients of high bioavailability. The unique contribution of fish as an important micronutrient source in Burma is compromised by the lack of nutrient data. Data on nutrient intakes from fish point to its crucial role in the diet in a context where levels of malnutrition remain persistently high, contributing a significant share of protein, dietary iron, calcium and Vitamin B12 requirements. Withstanding under-represented nutrient contribution results, the value of fish providing protein and micronutrients is still demonstrated in this analysis. By disaggregating nutrient contribution at the species level, we are able to demonstrate the importance of species diversity and the need for nutrition-sensitive approaches and monitoring trends in household fish consumption among the population and geographical groups described.

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TABLES AND FIGURES

Table 1. Categorization of commonly consumed fish species

Burmese Name	Common Name	Scientific Name
Freshwater Capture Species		
Ngayut/Ngayant	Striped snakehead	*Channa striata*
Ngakha/Ngakhu	Walking catfish	*Clarias batrachus*
Ngagyee	Stinging catfish	*Heteropneustes fossilis*
Ngapyamya	Climbing perch	*Anabas testudineus*
Ngaton/Ngamyinn	Pangasius/silondia	*Pangasius pangasius/Silona silondia*
Ngathalauk	Hilsa	*Tenualosa ilisha*
Fish meat	Rohu, bronze featherback, Indo-pacific king mackerel	*Labeo rohita, Notopterus notopterus, Scomberomorus guttatus*
Other small river fish		*Puntius chola, Parambasis ranga, Mystus vittatus, Salmostoma sardinella*
Other medium river fish		*Labeo stolizkae, Mystus cavasius, Osteobrama belangeri, Oreochromis niloticus*
Other large river fish		*Catla catla, Wallago attu, Rita rita*
Kakatit	Barramundi	*Lates calcarifer*
Marine Capture Species		
Ngamoke	Silver pomfret	*Pampus argenteus*
Ngashwe	Yellow pike conger	*Congresox talabon*
Ngapokethin	Panna croaker	*Pennahia microcephalus*
Sardine		*Sardinella gibbosa/Dussumeiria elopsoides/Sardinella longiceps/Sardinella melanura/Sardinella albella*
Pazun Kywat	Marine shrimp	*Penaeus monodon/Penaeus merguiensis/Metapenaeus affinis/Parapenaeopsis stylifera*
Pazun Doke	Giant freshwater prawn	*Macrobrachiu rosenbergii*
Squid and like sea fishes	Squid and cuttlefish	*Sepia aculeate/Sepia pharaonic/Uroteuthis duvaucelii/Uroteuthis chinensis/Ancistroncheirus lesueuri*
Other small sea water fish	Anchovy, thyrssa	*Setipinna taty/Stolephorus indicus/Stolephorus commersonii/Cailia ramcarati/Coilia dussumieri, Thryssa setirostri/Thryssa hamiltonii/Thryssa mystax/Thryssa baelama*
Other medium sea water fish	Grey mullet, mango fish, smallhead hairtail, threadfin bream	*Mugil cephlus, Polynemus paradise, Eupleurogrammus muticus, Nemipterus bathybius*
Other large sea water fish	Talang queenfish	*Scomberoides commersonnianu*
Farmed (Aquaculture) Species		
Ngamyitchin	Rohu	*Labeo rohita*

Burmese Name	Common Name	Scientific Name
Ngagyin	Mrigal	*Cirrhinus mrigala*
Dried/Processed Species		
Nga Yantchauk	Dried striped snakehead	*Channa striata*
Other dried small river fish		*Puntius chola, Mystus cavasius*
Other dried medium river fish		*Heteropneustes fossilis, Clarias batrachus*
Ngakunshutu chauk	Mackerel	*Rastrelliger kanagurta*
Other dried small sea water fish		*Septipinna taty/Stolephorus indicus/Stolephorus commersonii/Coilia ramcarati/Coilia dussumieri/Thryssa setirostri/Thryssa hamiltonii/Thryssa mystax/Thryssa baelama*
Other dried medium sea water fish		*Eupleurogrammus muticus*
Dried prawns		Mixed of averaged groups
Shrimp paste		
Fish/shrimp paste		Averaged group of best available matches; Local recipes and samples required
Ngapiyae	Fish sauce (liquid fermented fish)	
Nagpikaung	Salted fish	*Osteobrama belangeri, Scomberoorus guttatus*

Table 2. Consumption in 2005 and 2010 for wild capture freshwater and marine fish product categories, as well as farmed fish

	Consumption in 2005 (in kg)	Consumption in 2010 (in kg)
Freshwater Capture	10.57	6.83
Marine Capture	4.23	3.09
Aquaculture	3.83	3.38

Table 3. Consumption in 2005 and 2010 for each processed fish product category. There were no reported processed fish products from aquaculture.

	Consumption in 2005 (in kg)	Consumption in 2010 (in kg)
Dried/processed Freshwater	4.22	3.00
Dried/processed Marine	5.56	4.42

Table 4. Average regional consumption in Burma in 2005 and 2010. The last row ("Overall") indicates the average consumption for the entire country

Region	Consumption in 2005 (in kg)	Consumption in 2010 (in kg)
South	33.010	25.50
Lower	38.699	26.80
West	25.053	22.29
Central	18.594	13.75
North	23.705	16.53
East	11.747	9.71
Overall	25.135	20.72

Table 5. Consumption by wealth quintile

	Consumption in 2005 (in kg)	Consumption in 2010 (in kg)
Quintile 1	14.638	7.57
Quintile 2	26.288	11.73
Quintile 3	34.349	16.30
Quintile 4	41.935	21.04
Quintile 5	57.435	28.22

Table 6. Consumption by rural vs. urban

	Consumption in 2005 (in kg)	Consumption in 2010 (in kg)
Rural	26.853	21.11
Urban	27.925	20.58

Table 7. List of states within each region

North	Central	West	East	Lower	South
Kachin	Magway	Chin	Shan	Bago	Kayin
Sagaing	Mandalay	Rakhine	Kayah	Ayeyarwady	Mon
				Yangon	Tanintharyi

Table 8. Nutrient contributions from all fish and fish products. Based on 2010 fish consumption data from IHLCA survey. Recommended per day nutrient intake values come from dietary reference intakes (DRIs) for a person 19-50 years of age estimated by the Office of Disease Prevention and Health Promotion (HHS and USDA 2015). RDA refers to the Recommended Dietary Allowance (RDA), which is the average daily level of intake sufficient to meet the nutrient requirements of nearly all healthy people (HHS and USDA 2015). Data on all micronutrients other than Iron, Zinc, Calcium, B12 need to be considered cautiously as there were many species for which nutrient data were not available. As a result, the contribution of fish to micronutrient intakes will be underestimated for these particular nutrients.

Nutrient	Per Day Total Nutrient Contribution	Recommended Per Day Nutrient Intake (Women)	Recommended Per Day Nutrient Intake (Men)	Nutrient Intake from Fish as Share of RDA (Women/Men)
Energy (kcal)	81.5	1900	2550	4.29%/3.20%
Energy (kJ)	241.9			
Protein (g)	9.8	46	56	21.30%/17.5%
Fat (g)	1.7	65	80	2.62%/2.19%
Iron (mg)	4.4	18	8	24.44%/55%
Zinc (mg)	0.7	8	11	8.75%/6.36%
Calcium (mg)	243.3	1000	1000	24.33%
Vitamin B12 (µg)	1.2	2.4	2.4	50%
Sodium (mg)	900.7	2300	2300	39.16%
Iodine (µg)	21.0	700	700	3%
Selenium (µg)	11.9	55	55	21.64%
Magnesium (mg)	16.0	315	410	5.08%
Copper (mg)	4.4	900	900	0.49%
Phosphorus (mg)	106.9	700	700	15.27%
Potassium (mg)	127.6	4700	4700	2.71%
Manganese (mg)	0.0	1.8	2.3	0%
Vitamin D3 (µg)	0.9	600	600	0.15%
Vitamin E (mg)	0.1	15	15	0.67%

Table 9. Nutrient contributions from all marine fish and fish products. Based on 2010 fish consumption data from IHLCA survey. Recommended per day nutrient intake values come from dietary reference intakes (DRIs) for a person 19-50 years of age estimated by the Office of Disease Prevention and Health Promotion (HHS and USDA 2015).

Nutrient	Per Day Total Nutrient Contribution	Recommended Per Day Nutrient Intake (Women)	Recommended Per Day Nutrient Intake (Men)
Energy (kcal)	30.3	1900	2550
Energy (kJ)	87.4		
Protein (g)	3.6	46	56
Fat (g)	0.5	65	80
Iron (mg)	1.7	18	8
Zinc (mg)	0.2	8	11
Calcium (mg)	76.9	1000	1000
Vitamin B12 (µg)	0.2	2.4	2.4
Sodium (mg)	367.5	2300	2300
Iodine (µg)	5.5	700	700
Selenium (µg)	5.5	55	55
Magnesium (mg)	6.6	315	410
Copper (mg)	3.2	900	900
Phosphorus (mg)	56.1	700	700
Potassium (mg)	46.0	4700	4700
Manganese (mg)	0.0	1.8	2.3
Vitamin D3 (µg)	0.2	600	600
Vitamin E (mg)	0.0	15	15

Table 10. Nutrient contributions from all freshwater fish and fish products. Based on 2010 fish consumption data from IHLCA survey. Recommended per day nutrient intake values come from dietary reference intakes (DRIs) for a person 19-50 years of age estimated by the Office of Disease Prevention and Health Promotion (HHS and USDA 2015).

Nutrient	Per Day Total Nutrient Contribution	Recommended Per Day Nutrient Intake (Women)	Recommended Per Day Nutrient Intake (Men)
Energy (kcal)	37.7	1900	2550
Energy (kJ)	98.5		
Protein (g)	4.0	46	56
Fat (g)	0.8	65	80
Iron (mg)	0.4	18	8
Zinc (mg)	0.3	8	11
Calcium (mg)	85.0	1000	1000
Vitamin B12 (µg)	0.7	2.4	2.4
Sodium (mg)	17.6	2300	2300
Iodine (µg)	3.2	700	700
Selenium (µg)	5.4	55	55
Magnesium (mg)	6.0	315	410
Copper (mg)	0.0	900	900
Phosphorus (mg)	28.6	700	700
Potassium (mg)	54.5	4700	4700
Manganese (mg)	0.0	1.8	2.3
Vitamin D3 (µg)	0.6	600	600
Vitamin E (mg)	0.1	15	15

Table 11. Nutrient contributions from all farmed fish and fish products. Based on 2010 fish consumption data from IHLCA survey. Recommended per day nutrient intake values come from dietary reference intakes (DRIs) for a person 19-50 years of age estimated by the Office of Disease Prevention and Health Promotion (HHS and USDA 2015).

Nutrient	Per Day Total Nutrient Contribution	Recommended Per Day Nutrient Intake (Women)	Recommended Per Day Nutrient Intake (Men)
Energy (kcal)	7.0	1900	2550
Energy (kJ)	29.3		
Protein (g)	1.4	46	56
Fat (g)	0.1	65	80
Iron (mg)	0.2	18	8
Zinc (mg)	0.1	8	11
Calcium (mg)	51.8	1000	1000
Vitamin B12 (μg)	0.4	2.4	2.4
Sodium (mg)	1.5	2300	2300
Iodine (μg)	1.3	700	700
Selenium (μg)	1.7	55	55
Magnesium (mg)	2.7	315	410
Copper (mg)	0.0	900	900
Phosphorus (mg)	5.1	700	700
Potassium (mg)	22.0	4700	4700
Manganese (mg)	0.0	1.8	2.3
Vitamin D3 (μg)	0.1	600	600
Vitamin E (mg)	0.0	15	15

Table 12. Nutrient contributions from all dried and processed fish products. Based on 2010 fish consumption data from IHLCA survey. Recommended per day nutrient intake values come from dietary reference intakes (DRIs) for a person 19-50 years of age estimated by the Office of Disease Prevention and Health Promotion (HHS and USDA 2015).

Nutrient	Per Day Total Nutrient Contribution	Recommended Per Day Nutrient Intake (Women)	Recommended Per Day Nutrient Intake (Men)
Energy (kcal)	26.9	1900	2550
Energy (kJ)	112.6		
Protein (g)	4.6	46	56
Fat (g)	0.7	65	80
Iron (mg)	4.0	18	8
Zinc (mg)	0.3	8	11
Calcium (mg)	132.5	1000	1000
Vitamin B12 (μg)	0.3	2.4	2.4
Sodium (mg)	876.4	2300	2300
Iodine (μg)	15.4	700	700
Selenium (μg)	4.2	55	55
Magnesium (mg)	6.8	315	410
Copper (mg)	4.4	900	900
Phosphorus (mg)	64.2	700	700
Potassium (mg)	52.5	4700	4700
Manganese (mg)	0.0	1.8	2.3
Vitamin D3 (μg)	0.2	600	600
Vitamin E (mg)	0.0	15	15

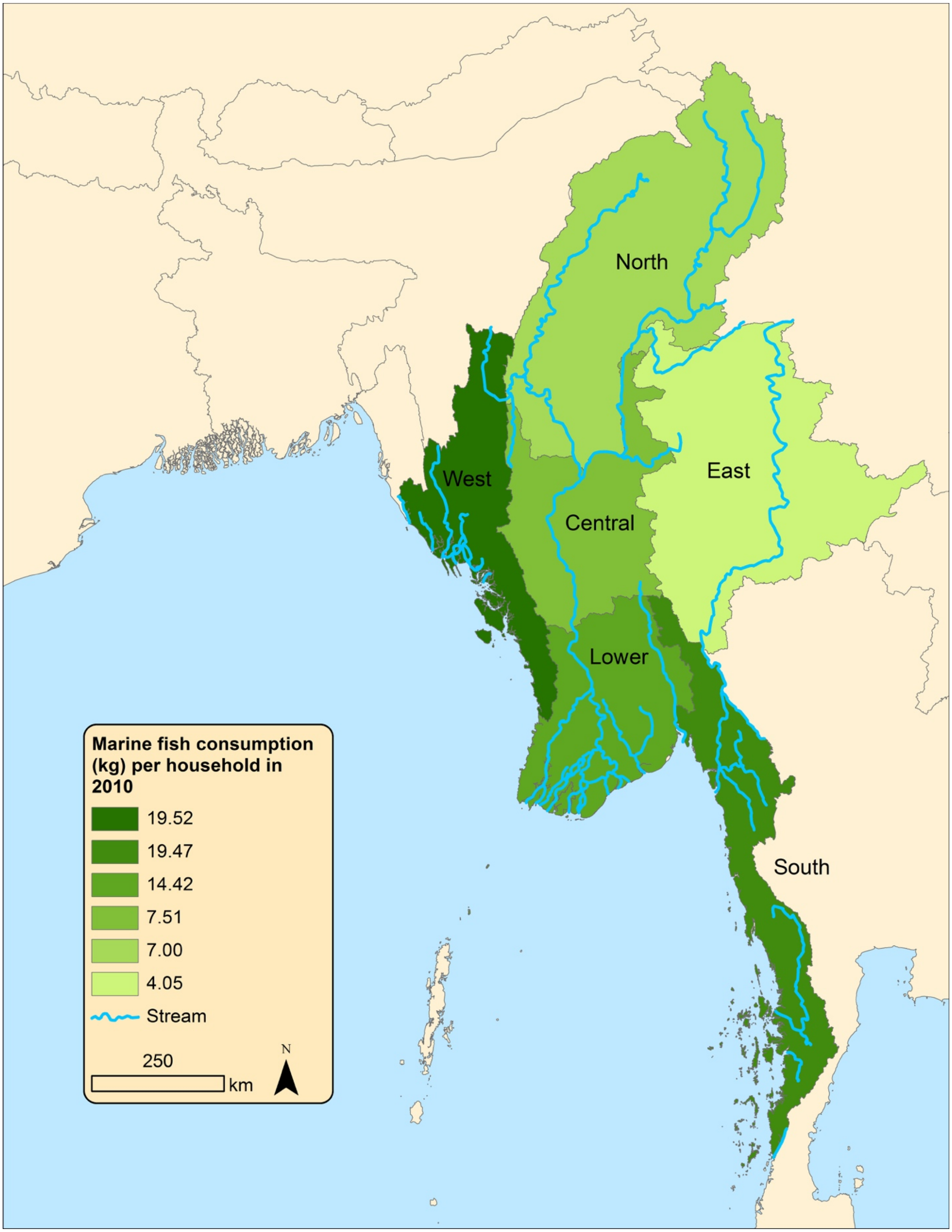

Figure 1. Map illustrating variation, by zone, of marine fish consumption (kg) per household in 2010.

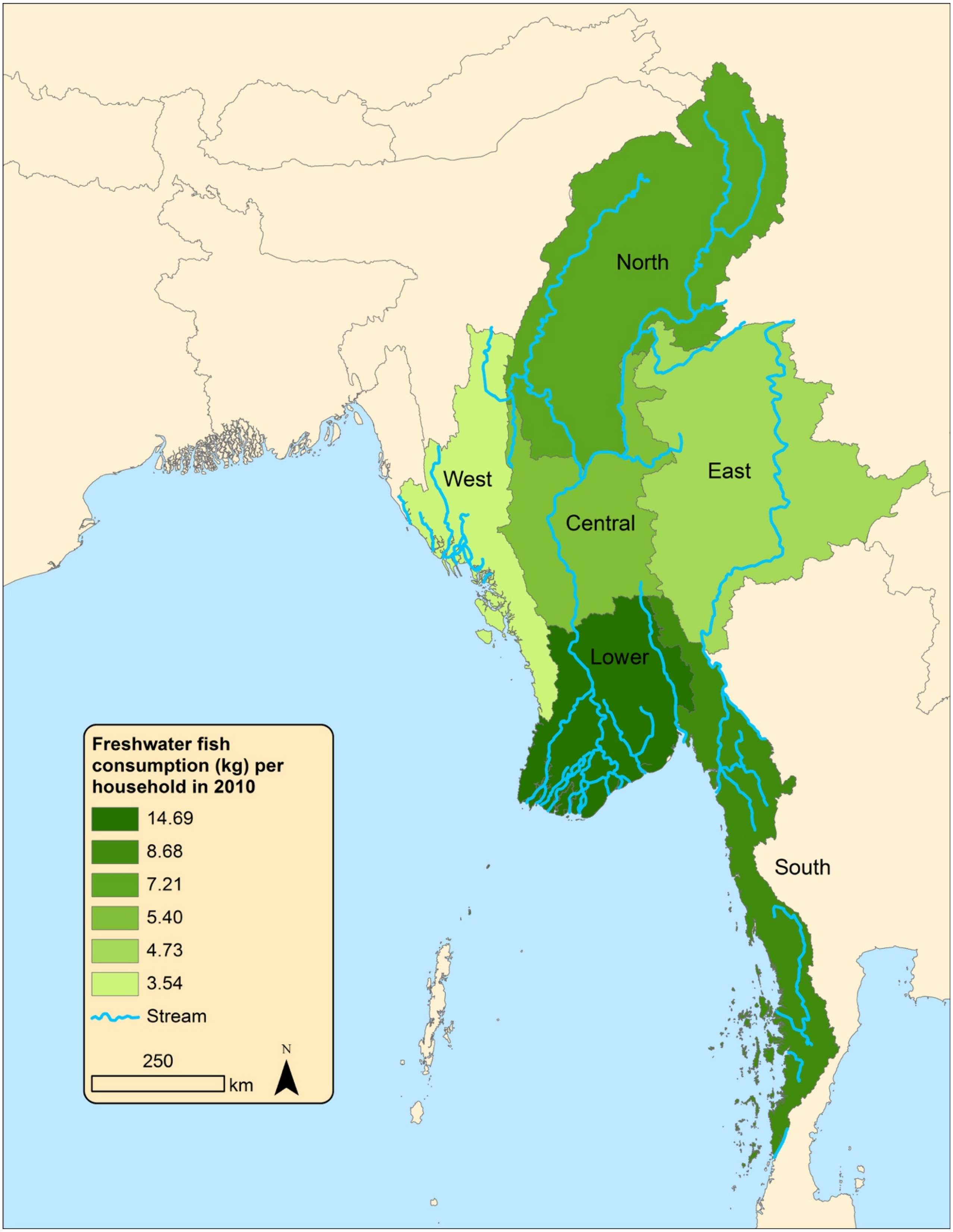

Figure 2. Map illustrating variation, by zone, of freshwater fish consumption (kg) per household in 2010.

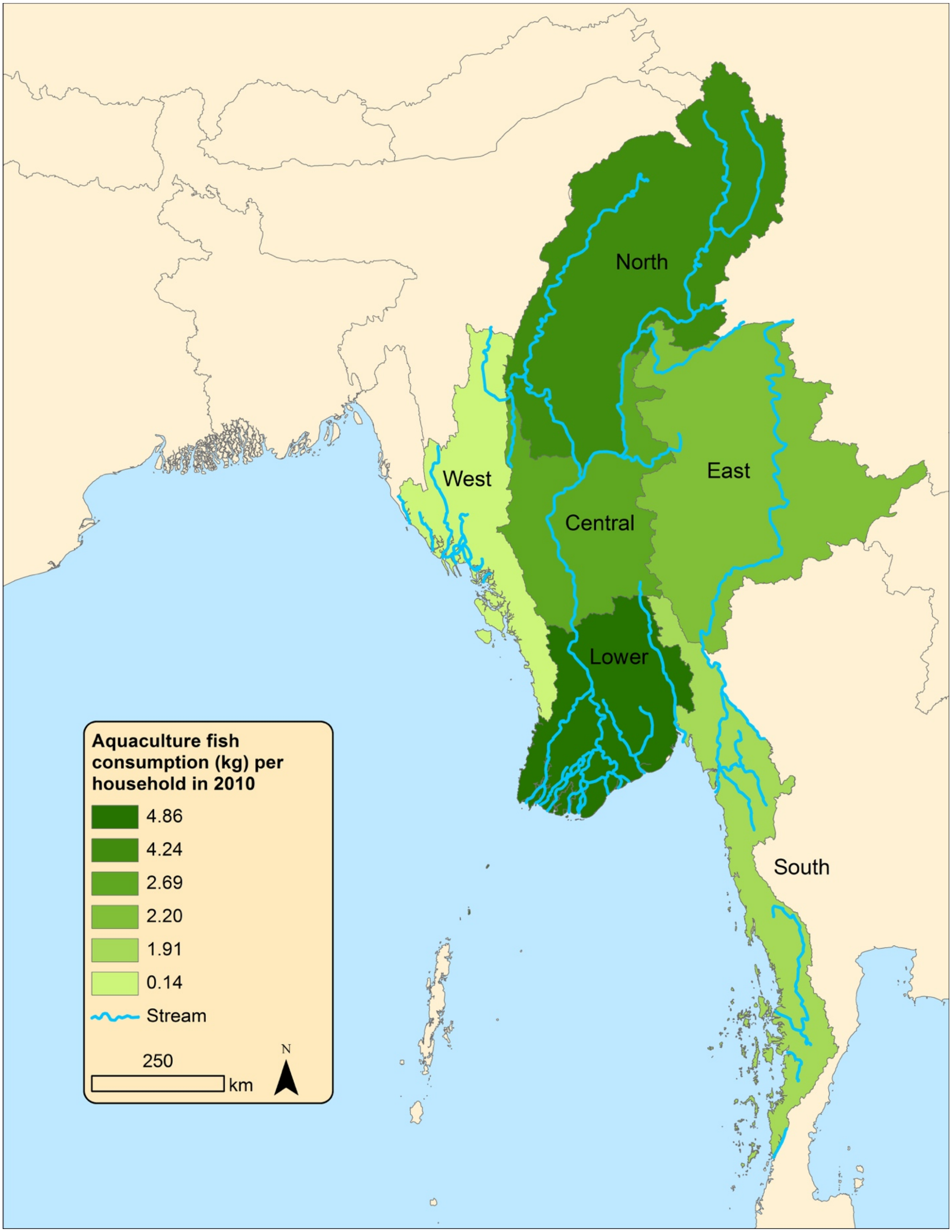

Figure 3. Map illustrating variation, by zone, of farmed (aquaculture) fish consumption (kg) per household in 2010.

TOPIC AREA

FOOD SAFETY, POST-HARVEST, AND VALUE-ADDED PRODUCT MANAGEMENT

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ENHANCING FOOD SECURITY AND HOUSEHOLD NUTRITION OF WOMEN AND CHILDREN THROUGH AQUACULTURE AND CAPTURE FISHERIES IN CAMBODIA AND VIETNAM IN THE DRY SEASON- PART I

Food Safety, Post Harvest, and Value-Added Product Management/Study/16FSV01UC

Touch Bunthang[1], So Nam[1], Cheng Phen[1], Tran Minh Phu[2], Huynh Van Hien[2], Tran Thi Thanh Hien[2], Hillary Egna[3], and Robert Pomeroy[4]

[1]*Inland Fisheries Research and Development Institute, Fisheries Administration, Phnom Penh, Cambodia*
[2]*College of Aquaculture and Fisheries, Can Tho University, Vietnam*
[3]*College of Agricultural Science, Oregon State University, OR, USA*
[4]*Department of Agricultural and Resource Economics/CT Sea Grant, University of Connecticut, CT, USA*

ABSTRACT

Fish is an integral part of women and preschool children's staple food for their daily consumption and is a main source of protein and other key micronutrients. At the same time, the prevalence of malnutrition among women and preschool children continues to be a major problem in Cambodia. However, data and information on the commonly consumed fish species and the current dietary intake of women and preschoolers during the dry season in Cambodia are lacking. This study aimed to identify commonly consumed fish species and Other Aquatic Animals (OAAs); evaluate food consumption, dietary energy, and nutrient intakes among women and preschool children during the dry season. Three hundred women and 327 preschoolers (aged 6 months to 5 years) were randomly selected for study in Cambodia. One hundred and fifty of the eligible women and 150 of the eligible preschool children were randomly selected for study in Vietnam. The 24hr-food recall questionnaire was used. Microsoft Excel 2013 and SPSS Statistics Version 20.0 were used for data entry and analysis. Forty-three (43) fish species and OAAs were consumed by Cambodian women, and 35 fish species and OAAs were consumed by Cambodian preschoolers. Fish provides 75.4% and 72.5% of the total animal protein intake to Cambodia women and preschool children, respectively. Cambodian women consumed fish at 137.4 g/day and preschoolers at 49.1g/day. Cambodian women's total average daily per-capita food consumption was 835.6 g/day, while for preschoolers it was 454.2 g/day. Cambodian women's total average dietary energy intake was 1917.5 kcal/day, while for preschoolers it was 780.8 kcal/day. The overall mean one day per-capita food consumption rate of Vietnamese women and preschool children was 750 g/p/day (1411 Kcal) and 638 g/p/day (764 Kcal), in raw-as-purchased form, respectively. Rice was highly consumed by both Vietnamese women and preschool children at 230g/p/day and 153 g/p/day, respectively, accounting for 30.7% and 22.5% of daily food consumption, respectively. Vegetables and fish were the second top food group commonly consumed by women in the study, at 16.3% and 16.9%, respectively. Vietnamese women consumed 21 fish species, OAAs, and their products, with 127 g/day contributing nearly 16.9% of total diet intake. For the women's diet, fish and fish products are the major contributors of protein at 41.71%,

fats at 22.15 %, iron at 9.53%, calcium at 40.7%, and vitamin A at 25.39%; while for preschool children, milk and milk products play an important role in nutrient intake.

Keywords: 24hr-food recall, Food Consumption, Macronutrient intakes, Women, and Preschool Children, Cambodia and Vietnam

INTRODUCTION

Fish is an integral part of women and preschool children's staple food for their daily consumption and is a main source of protein and other key micronutrients. At the same time, the prevalence of malnutrition among women and preschool children continues to be a major problem in Cambodia.

During the previous phase of the AquaFish Innovation Lab (2013-2015), the study of food and nutritional consumption by women and children in Cambodia was conducted during the rainy season under the University of Connecticut Investigation 13HHI02UC. The recommendation provided from the final national workshop on 26 September 2015 at IFReDI in Phnom Penh is that the study of food and nutritional consumption should be conducted in the dry season in Cambodia in order to cover the whole year.

There are two major seasons in the Lower Mekong Basin region, and they bring about changes in people's food consumption patterns. During the wet season, there is an abundance of fish, while during dry season there is less fresh fish available and people use more processed fish products. This study will allow us to make comparisons of food consumption patterns between the seasons to better assess ways to improve nutrition for women and children. Moreover, information about consumption of fish and OAAs from capture fisheries and aquaculture by women and children in the Mekong Delta of Vietnam is limited.

OBJECTIVES

The primary purpose of this activity was to identify the commonly consumed fish species and OAAs of the sample women and preschool-age children. The secondary purpose was to evaluate their current food consumption: energy, and key nutrient intakes of women and preschool children and the relative contribution made by fish and OAAs and its products to total nutrient intake of studied subjects in Cambodia and Vietnam.

MATERIALS AND METHODS

Study Design and Scope

Stung Treng province (Upstream Mekong River); Prey Veng province (Downstream Mekong River); and Kampong Thom province (Tonle Sap Area) in Cambodia and Long Xuyen city, Châu Phu town and Tan Chau town in Vietnam were selected for study sites. The data collection was conducted during the dry season (January to May 2017) in Cambodia and Vietnam. The target subjects of the study were women and preschool children (aged 6 months to 5 years). Three hundred (300) eligible women and 327 eligible preschool-age children in Cambodia, and 150 eligible women and 150 eligible preschool–age children in Vietnam, were selected by using simple randomized sampling. Dietary intake was surveyed through face-to-face interviews by using a single 24-hour food recall to estimate the amount of food that had been eaten in the past 24 hours. Food models were used to identify food items that were eaten by the subjects. All food and beverages consumed were recorded using standard household measurement and an electronic scale (precision to 0.1g). The names of local dishes consumed were also recorded. The amount of each food item consumed was estimated from the real food models. Mothers were asked to show the amount of food consumed by her child, who was then weighed. All food item consumption by women and preschoolers were converted to weight in grams, and the nutrient content of the foods consumed were computed by using the ASEAN Food Composition Table (ASEANFCT, 2000). Nutrients for evaluation included: energy; macronutrients (protein, carbohydrate, and fat); and key micronutrients (iron, zinc, calcium, and vitamin A). The nutrient intakes of women and preschool children were then compared to the Recommended Dietary

Allowances Harmonization in Southeast Asia (Barba, 2008) to determine the level of nutritional adequacy of the food intake to estimate the amount of food that had been eaten.

Training and Survey Organization

Data were collected by eight trained field enumerators - four enumerators from Cambodia and four from Vietnam. The training aimed to educate field enumerators on how to conduct dietary assessments by using the 24-hour food recall questionnaire; to educate the interviewers to be familiar with fish species; to reinforce the recall interviewers with exercise practices and pilot testing; and to educate the interviewers to be familiar with the questionnaire before data entry was performed. Pilot testing of questionnaires was conducted in order to identify potential problems in the questionnaires, the questions, and the recall form.

A letter of survey objectives was presented to the local authorities informing them of the survey before the actual field work was started. Questionnaires were cross-checked by members of the team for any missing pieces of information, followed by data entry. Microsoft Excel 2013 and SPSS Statistics Version 20.0 were used for data entry and analysis. Data coding, cleaning, and cross-checking were then conducted. Descriptive statistics were used.

RESULTS

Food Consumption in Cambodia: By Women

Food consumption in different areas of the country may be influenced by topography, religious customs, cultural relationships, trades and price (food affordability), agricultural products (local food availability), family size, and household food distribution. These factors influence the food consumption patterns of individuals, especially for women and children. The majority of foods that made up the sample women's diet came from a plant source (77%), while 23% came from an animal source (Figure 1).

The overall sampled women's diet is basically a rice-vegetable-fish combination (Figure 2). In terms of weight, the total mean one day per-capita food consumption is 835.6g/d, in as-purchased raw form. The most highly consumed food was cereal and cereal products at 359.4 g/d (42.9% of the total food intake). Vegetables were the second largest food group commonly consumed by sample women at 148.9 g/d (17.8 % of the total food intake). Intake of fish, and OAAs and their products were the third largest food group commonly consumed at 137.4 g/d (16.4% of the total food intake). Consumption of fruits and beverages was 54.5 g/d (6.5%). The rest of the major food groups consumed in small amounts were fats and oils, eggs, sugar and syrups, legumes, nuts, and seeds, and starch roots and tubers.

Among the studied provinces, the Prey Veng province, located in the Lower Mekong region of Cambodia, showed the highest food intake at 843.9g/d, lower consumption compared to the rainy season which was 884 g/d, followed by Kampong Thom Province, Tonle Sap area, at 830.8g/d, compared to the rainy season consumption which was 855 g/d, while Stung Treng province, located in the Upper Mekong region of Cambodia, was the lowest at 823.2 g/d, compared to the rainy season consumption which was 843 g/d (Table 7).

Intake of cereals and cereal products, predominantly rice, was high in Prey Veng province, at 365.9g/d, followed by Kampong Thom province at 355.2g/d. Stung Treng province consumed 356.1 g/d. Kampong Thom province consumed vegetables at around 150.5 g/d, followed by Prey Veng province at around 149g/d and Stung Treng was around 147.3g/d. Fish, and OAAs and their products were generally eaten more than meat, poultry, or eggs in all sample provinces. Higher consumption of fish was observed in Kampong Thom province at around 140.5g/d, followed by Stung Treng province at around 139.1g/d, and Prey Veng province at 132.7g/d. The other different food groups, fats and oils, eggs, sugar and syrups, legumes, nuts, and seeds, and starch roots and tubers were also consumed by sample women across the studied provinces.

By Pre-School Children

Diets of sampled pre-school children came from plant sources (76%) and animal sources (24%) (Figure 3). The mean one-day total food intake of children aged 6 months to 5 years was 454.2g, in as-purchased form (Table 2, Figure 4), consisting of 229.5 g (50.5%) of cereal and cereal products, of which rice and rice products were the predominant forms; 45.2g (9.9%) of milk and milk products; 49.1g (10.8%) of fish and OAAs; and a combination of vegetables and fruits at 48.1g (10.6%).

Energy-giving foods such as rice, and body-building foods such as milk, fish, meat, poultry, and eggs, are food items that are needed most importantly in the diet of preschool children to support their fast rate of growth and development. Rice contributed more than half of the total food intake. This age group's intake of fish and fish products was about 10.8%, while intake of meat and meat products was 2.2 %, and intake of poultry and eggs was 1.7 %. Milk and milk products were at 9.9% of the total food intake. Fruits and vegetables amounted to 19.5 and 28.6 g, respectively, which accounted for 4.3% and 6.3% of total food intake, respectively. The intake of fats and oils, at 0.9 g or 0.2% of the total food intake, although low, is important for the transport of fat-soluble vitamins, especially vitamin A found in animal tissues, or beta-carotene, the precursor of vitamin A from plant tissues.

Table 2 shows the mean one-day food intake of children aged 6 months to 5 years by province. Prey Veng province was the highest at 461g/d, lower consumption compared to the rainy season intake of 547g/d. Stung Treng province was at 456g/d, compared to the rainy season consumption which was 467g/d. The lowest food intake was observed in Kampong Thom province at 444.6g/d, compared to the rainy season consumption, which was 458g/d.

Intake of cereals and cereal products, predominantly rice, in the dry season was high in Stung Treng province at 245.5g, followed by the mountainous Prey Veng province around 227.5g per day, while the lowest intake was observed in Kampong Thom province at around 215.6g/d. Fish and beverages were the second largest items consumed across the studied provinces, with the combination of both items accounting for nearly one-fourth of total food intake by pre-school children. Milk and milk products ranked third across the studied provinces, with Prey Veng province at 48.9 g (10.6%), followed by Kampong Thom province at 47.5 g (10.7%) and Stung Treng province, which had the lowest consumption at 39.1 g (8.6%). Food groups such as fruits, vegetables, eggs, sugar and syrup, starchy roots and tubers, legumes, nuts and seeds, and beverages were also consumed by this age group.

During the dry season, women consume 40 fish and OAA species with an average consumption of 137.4g/d, compared to the rainy season, which was 145.3g/d. The most consumed fish species in terms of weight and percent contribution by women consuming fish were Trey Riel (*Cirrhinus sp.*) at 30g (21.7%) of total fish intake per day; Trey Ros (*Channa striata)* at 18.2g/d (13.2%); and Trey Chrakeng (*Puntioplites proctozystron*) at 14 g/d (10.1%), (Table 3).

During the dry season, preschoolers consumed 35 fish and OAA species, with an average consumption of 49.1g/d, compared to the rainy season at 53g/d. The most consumed fish species in terms of weight and percent contribution by children were Trey Riel (*Cirrhinus sp.*) at 11.9g/d (24.1%); Trey Ros (*Channa striata*), 9.2g/d (18.6%) and Trey Changwa Phlieng (*Esomus longimanus*), 4.5 g/d (9.1%), (Table 4).

Nutrient intake in Cambodia: By women

All major food groups contribute to the caloric intake of individuals, especially women and children; therefore, caloric and protein intake measured against the recommended daily allowance is a good indicator of food adequacy. An intake of 1g of carbohydrate-rich foods such as cereal and its products, starchy tubers and roots, sugar and syrups, or fruits and vegetables will generate 4 Kcal. An intake of 1g of protein-rich foods such as fish, meat, milk, poultry, eggs, dried beans, and nuts, seeds, and their products will also generate 4 Kcal. An intake of 1g of fats and oils, including butter or margarine, will give 9 Kcal. The sources of calories in a diet, namely carbohydrates, proteins, and fats are an indicator of the quality of the diet.

The diet of women sampled in this study had a total energy of 1917.5Kcal, compared with women's diet during the rainy season was 1976 Kcal, the largest amount of which came from carbohydrates at 69%, with desirable contribution from proteins at 14%, and low contribution from fats and oils at 17% (Figure 5).

The total energy and nutrient intake among the three provinces ranked from 1780.7 Kcal (Kampong Thom) to 1987.6 Kcal (Prey Veng), (Table 5). The sampled women's daily protein intake ranged from 60.8 to 65.8 g, carbohydrate intake from 338.5 to 354.6g, while lipid intake ranged from 28.4 to 42g. Iron intake ranged from 11.4 to 12.6 mg, zinc from 3 to 3.9 mg, calcium intake from 467.1 to 591.2 g, and vitamin A from 386.5 to 518.9mcg RE.

In terms of energy and nutrient adequacy, half of the sampled women met at least 100% of their recommended intake for energy. About 70% of the sampled women met at least 80% of the recommended intake of protein. Less than one-fifth of the sample women met at least 80% of the recommended intake for iron. More than one-third of sampled women met at least 80% of the recommended intake for zinc. Close to one-fourth of sampled women met the recommended intake for calcium, and more than one-fourth of sampled women met the recommended intake for vitamin A (Table 5).

By preschoolers

The total energy intake of sampled pre-school children among the three provinces ranked from a low of 757.7 Kcal (Stung Treng) to a high of 805.2 Kcal (Kampong Thom), (Table 6). The sampled preschool children's daily protein intake ranged from 26.1 to 27.8 g, carbohydrate intake from 116.2 to 129.2g, and lipid intake from 12.7 to 16g.

Iron intake ranged from 4.6 to 4.8mg, zinc intake from 1.2 to 1.8mg, calcium intake from 202.7 to 281g, and vitamin A intake from 187.2 to 227.9mcg RE. Among the preschool children, only 27% met the recommended dietary intake for energy, less than half met at least 80% of protein requirements, and close to one-fourth of the preschool children met at least 80% of recommended intake for iron. Less than one-tenth of the preschool children met at least 80% of recommended intake for zinc, about 17% met the recommended intake for calcium, and more than 18% met the recommended intake for Vitamin A.

The contribution of particular food groups to total energy and nutrients intake indicates that cereal and cereal products are the top energy contributors in the diet of sampled women, contributing 59.2% (Table 7). Because of the large bulk of this food group, it is also the major contributor of carbohydrates at 77.5%. It is also the second contributor of protein at 30%, fats at 14.4%, iron at 43.1%, calcium at 21.4%, and vitamin A at 7.6%. Fish and fish products are the major contributors of protein at 47.5%, fats at 27.6%, and iron at 25.8%. Meat and meat products follow fish and fish products in their contribution to the total nutrient intake. Vegetables and fruits, on the other hand, are the second largest contributor of carbohydrates at 7.9%, and are the major contributors of iron, zinc, calcium and vitamin A. Fruits were the top contributor to total vitamin A intake, while beverages were the second largest contributor to total energy intake by the sampled women.

The contribution of different food groups to total energy and nutrients intake indicates that cereal and cereal products are the largest contributors in the sampled pre-school children's diet to energy, carbohydrates, and iron at 53.4%, 73.5%, and 44.5%, respectively (Table 8). It is also the second contributor to protein and fats at 34.6% and 21.5%, respectively.

Fish and fish products are top contributors to protein and fats with 40.8% and 28.4%, respectively. Milk and milk products were the largest contributors to zinc and calcium at around 39.9% and 28.4%, respectively. Fruits and vegetables, on the other hand, were the major contributor to vitamin A, while the other food groups, such as starchy roots and tubers, legumes, nuts and seeds, sugars and syrups, beverages, and condiments and spices were lesser contributors to energy and nutrient intake by pre-school children.

Role of fish in nutrition security in women and preschool children in Cambodia

Fish plays a great role in meeting dietary energy needs. When energy needs of the body cannot be supplied by rice and other carbohydrate-rich foods, the body metabolizes the protein from fish to sustain the caloric need of the body for the proper functioning of various physiological and biochemical processes, such as digestion and metabolism of foods to sustain life. Fish is also a source of fats. The fats and the protein contributed by fish significantly mitigate caloric deficiency in women's and preschool children's diets, and perhaps may mitigate protein-energy malnutrition, which is the form of malnutrition that exists in developing countries like Cambodia.

Fish contribute some fat, but overall intake of fat was low - it is far lower than the desirable contribution of fats to total dietary energy, recommended at 30-40% for infants and 20-30% for all others. The low-fat intake is one reason for low calorie intake. Fish provides protein, which the body uses for optimal growth, but in the context of low calorie intake the body must turn to protein to satisfy energy needs. This is the form of protein-calorie malnutrition (Marasmus) and the major reason for stunted growth and development in Cambodia.

Another form of protein-calorie malnutrition (Kwashiorkor) is simple protein deficiency. This type is not common in Cambodia, which can be attributed to the availability of protein from fish. It can be noted that the contribution of protein for Cambodian women and pre-school children at 14% and 13%, respectively, to total dietary energy per capita meets the level of recommended protein at 10-15% (Figures 5 and 6).

Fish and OAAs and their products were the major contributors of energy and nutrients of animal origin for women, providing 75.4% of the total animal protein intake (Table 9). Meat, poultry, and eggs contributed less than one-fourth to the total animal protein intake, accounting for about 17% and 2.2%, respectively. Fish and OAAs and their products were also the major contributors of energy, fats, carbohydrates, iron, zinc, calcium, and vitamin A at 66%, 51.1%, 94%, 70.5%, 42.2%, 78.5% and 82.7%, respectively.

Fish and OAAs and their products were also the largest contributors to the total daily energy and nutrient intake from animal sources for preschool children, around 72.5% of the total animal protein intake (Table 10). Meat, poultry, and eggs contributed to the total animal protein intake at about 13.6% and 11.5%, respectively. Fish and OAAs and their products were also the major contributor to energy, fats, carbohydrates, iron, zinc, calcium, and vitamin A at 67%, 55.8%, 87.1%, 53.1%, 41%, 86.2.8% and 52.4%, respectively.

Food Consumption in Vietnam: By Women
Food consumption in different areas of Vietnam may be influenced by topography, religious customs, cultural relationships, trades and price (food affordability), agricultural products (local food availability); family size, and household food distribution. These and others are among the indicators that influence the food consumption patterns of individuals, especially women and children. The majority of foods that made up the sampled women's diet came from plants at 72.3%, while 24% came from animal sources, and 3.7% came from food groups such as condiments and spices (Figure 7). Figure 8 and Table 11 show that total sampled women's diet is basically a rice-vegetable-fish combination. In terms of weight, the total mean one day per-capita food consumption is 750 g/day, in raw-as-purchased form. The highlest consumed food was rice at 230g (30.7% of the total food intake), contributing to around one-third of the total food intake.

Vegetables and fish were the second top food group commonly consumed by sampled women, at around 122 and 127g, respectively (16.3 and 16.9% of the total food intake) per-capita per-day. There was also a high consumption of fruits at 52.2g (7%). The rest of the major food groups consumed in small amounts by the sampled women were fats and oils, beverages, eggs, sugar and syrups, and starch roots and tubers.

By Pre-School Children
The diet of sampled pre-school children came from animal sources accounting for 59.2%, plant sources contributing 38.9%, and 1.9% from condiments and spices (Figure 9). The mean one-day total food intake of children aged 6 months to 5 years was 683g, in as-purchased form, and consisted largely of 259.9 g (38%) of milk and milk products, the second largest intake was of 153 g (22.5%) of rice (mean food), and the third largest intake included cereals, meat and poultry, and fish and OAAs, at 9.6%; 9.1%, and 8.1%, respectively (Figure 10, Table 12).

Energy giving foods such as rice, and body building foods such as milk, fish, meat, poultry, and eggs, are food items that are needed most importantly in preschool children's diet to support the fast rate of growth and development. This age group's intake of milk and milk products was at 38% of the total food intake. Fruits and vegetables amounted to 22 and 20.6g, respectively, which accounted for about 3-3.2% of total food intake. The intake of fats and oils, at 0.7 g or 0.1% of the total food intake, although low, is important for the transport of fat soluble vitamins, especially vitamin A found in animal tissues, or beta-carotene, the precursor of vitamin A from plant tissues.

The study found that women consume 21 fish and OAA species, with an average consumption by women of 135.7g per-capita per-day. The 10 most consumed fish species in terms of weight and percentage contribution of sampled women consuming fish species per day is shown in Table 13. Snakehead (*C. striata*) was the highest species consumed at 39.1 g (28.8%) of total fish intake per day. Red tilapia (*Oreochromis sp.*) and Striped catfish (*P. hypophthalmus*) ranked second and third, at 26.2g (19.3%) and 17.6g (13%) of the total fish intake per day, respectively. Other fish species, aquaculture fish, and OAAs were also listed (Table 13).

Preschool children consumed 19 fish and OAAs species, with an average consumption is 67.1 g per-capita per-day. The 10 most consumed fish species in terms of weight and percentage contribution by children consuming fish species per day. Snakehead (*C. striata*) was also the largest consumption at 28.5 g (42.5%) of total fish intake per day. Red tilapia (*Oreochromis* sp.), swam eel *(M. albus*) and striped catfish (*P. hypophthalmus*) ranked 2nd, 3rd and 4th with 10.2g (15.2%), 8.00g (11.9%) and 7.4g (11%) to the total fish intake per day, respectively. Other fish species, aquaculture fish, and Other Aquatic Animals were also listed (Table 13).

Nutrient Intakes: The contribution of particular food groups to total energy and nutrients intake as shows in table 15, rice is top energy contributor in the sample women diet contributing at 56.17%. Because of the large bulk that this food group was eaten, it is also the major contributor of carbohydrates more than 70%. It is also the second contributor of protein at 24.62%, fats at 10.67%, iron at 39.71%, calcium 18.35% and Vitamin A 7.22%. Fish and fish products are the major contributors of protein at 41.71%, fats at 22.15 %, Iron at 9.53%, calcium at 40.7% and vitamin A at 25.39%. Meat and meat products follows fish and fish products in their contribution to the total nutrient intake. Fruits, vegetables and fish were the top contractor to total Vitamin A intake by the sample women. The contribution of different food groups to total energy and nutrients intake as shows in table 16. Milk and milk products are the largest contributors in the sample pre-school children's diet to protein, fat, calcium, zinc and Vitamin A. It is also the second contributor to energy and zinc at about 27% and 21.8%, respectively.

Rice is top contributor to carbohydrate and energy with 84.5% and 28.8%, respectively. Eggs, on the other hand, is the major contributor to vitamin A, while the other food groups such as starchy roots and tubers; sugars and syrup; beverages; condiments and spices were less contributors to energy and nutrient intake by pre-school children.

Table 17 shows that fish, OAAs and products was the major contributor of energy and nutrients to animal source, providing around two-third (61.8%) to the total animal protein intake. Meat and poultry, and eggs contributed around one-third to the total animal protein intake accounting about 28.6 % and 9.55 %, respectively. Fish, OAAs and products was also the major contributor to energy, carbohydrate, fats, iron, zinc, calcium, and vitamin A contributed at 44.2%, 98.1%, 34.6%, 34.5%, 47.2, 76.1%, and 76.8%, respectively.

For the preschool-age children, OAAs and products was the second largest contributor to the total daily energy and nutrient intake from animal food source which sharing around 44.68% to the total animal protein intake (Table 18). Meat, and poultry contributed the largest part to the total animal protein and energy intake which accounting for about 47.5 and 52.3%, respectively. Fish, OAAs and products was also the major contributor to carbohydrate, calcium, and vitamin A contributed at 44.2%, 78.3% and 20.57%. Eggs contributed largest portion in Vitamin A

DISCUSSION

Cambodia

Cambodian women's and preschool children's diets are basically a rice-vegetable-fish combination and have similar proportions: 77% from plant sources, and 23% from animal sources. The Philippine Food Consumption Survey in 2008 indicated that the Filipino diet comes 70% from plant sources, 29% from animal sources and 1% from condiments and prices. Our study in dry season and rainy season shows that Cambodian women's and preschool children's consumption of plant-source foods was higher than that of Filipinos, whereas consumption of animal-source foods was lower. Animal-source food in Cambodia is mainly from fish. Other animal sources like beef, chicken and pork are relatively expensive in Cambodia compared to fish which is available and affordable for rural households.

The overall mean daily per capita food consumption in dry season of women and preschool children are 853.6g and 454.2g compared to rainy consumption were at 861 g and 499 g in raw as-purchased form, respectively. By comparison, Filipino adult women from 13-19 years old consumed 709g and Filipino preschool children consumed 492g, of which milk and milk product contributed the largest amount at 188g; cereal and cereal products, accounting for 148g; fish and fish products at 36g; and vegetables 16g.

Fish and OAAs were the largest consumption by women and preschool children, estimated at 137.4g/d and 49.1g/d compared to rainy season consumed at 145.3 g and 52.9 g, respectively. Our findings are less than those in the study by Touch Bunthnag et al. (2011) at 172.7 g, perhaps because the surveys were conducted in different seasons.

Cambodian women's diet has a total energy of 1917.5 Kcal was lower than in rainy season at 1976 Kcal, the largest amount of energy (up to 69%) coming from carbohydrates, with a good contribution from proteins at 14%, but low contribution from fats and oils at 17%. Cambodian preschool children's diet has a total energy of 780.8 Kcal was lower than in rainy season 844.9 Kcal, of which carbohydrate generates the largest amount (70%), protein 13% and fats and oils 17%. The study conducted in Cambodia by FAO (2012) found that the contribution of total energy from carbohydrate was 76%, whereas the shares of protein and fat to the overall energy supply were 10% and 14%, respectively. A study by Phuong H Nguyen et al. (2013) in Vietnam showed that energy intake of Vietnamese women was 2196 kcal/day with 65.5%, 14.8%, and 19.5% coming from carbohydrate, protein and fat, respectively.

Fish, OAAs and their products were the major contributors of energy and nutrients of animal origin to women, providing 75.4% of the total animal protein intake, respectively; and also the major contributors of energy, fats, carbohydrate, iron, zinc, calcium, and vitamin A at 66%, 51.1%, 94%, 70.5%, 42.2%, 78.5% and 82.7%, respectively (Table 9). Again fish, OAAs and their products were the largest contributors to the total daily energy and nutrient intake from animal sources for preschool children, around 72.5% of the total animal protein intake and also the major contributor to energy, fats, carbohydrate, iron, zinc, calcium, and vitamin A at 67%, 55.8%, 87.1%, 53.1%, 41%, 86.2.8% and 52.4%, respectively (table 10). Another study by United Nation Children's Emergency Fund (UNICEF)/World Food Program (WFP) showed that fish is part of the daily diet of 74-80% of all children of over 11 months old. Fats and protein contributed from fish significantly mitigated caloric deficiency in women's and preschool children's diets and perhaps the protein-energy that exists in developing countries like Cambodia. Fish contribute some fat but overall intake of fat was low, far lower than the level of fats to total dietary energy recommended at 30-40% for infants and 20-30% for adults (RENI, 2002). The low fat intake is the reason for low calorie intake. Fish provides protein, which the body uses for optimal growth, but in the context of low calorie intake the body must turn to protein to satisfy energy needs. This form of protein-calorie malnutrition (Marasmus) is the major reason for stunted growth and development in Cambodia. Another form of protein-calorie malnutrition (Kwashiorkor) is simple protein deficiency. This type is not common in Cambodia and its rarity can be attributed to the availability of protein from fish. The contribution of dietary energy from protein, 14% for women and 13% for preschoolers, to total dietary energy per capita is in the recommended range of 10-15% of total protein intake per individual.

Aside from contributing to the total energy intake, fat of fish contains essential fatty acids namely, linoleic acid (omega 6), the precursor of arachidonic acid (ARA) and linolenic acid (omega 3), the precursor of DHA. These nutrients are not synthesized by the body, but rather must be obtained from food and are known to benefit health. DHA is a key component of the phospholipids in membranes of the eyes and brain, essential for brain and eye development infants and children. It reduces the risks of heart disease and stroke, prevents blood clots, lowers blood pressure, protects against irregular heartbeats, reduces inflammation, strengthens the immune system, and is essential for normal growth and development for healthy skin, normal growth and reproduction. A diet that is deficient in DHA is associated with poor growth, skin lesions, reproductive failure and fatty liver.

Beside its key contribution in meeting primarily protein and energy requirements, fish plays a significant role in meeting iron, zinc and vitamin A requirements in women and preschool children. Iron functions as part of hemoglobin, which transports oxygen in blood to cells and part of myoglobin in muscles, which makes oxygen available for muscle contraction. Iron is part of an enzyme in the immune system to help protect against infections and is involved in making amino acids, collagen, hormones or normal brain functions, for synthesis of neurotransmitters and brain growth in humans.

Overall, half of the sampled women met at least 100 % of their recommended intake for energy. About 70% of the sample women met at least 80% of the recommended intake of protein. Less than one-fifth of the sample women met at least 80% of the recommended intake for iron. More than 1/3 of sampled women met at least 80% of the recommended intake for zinc. Close to one-fourth of sampled women met the recommended intake for calcium and more than one-fourth of sampled women met the recommended intakes for vitamin A. In comparison, the Philippines Food Consumption Survey (2008) of adult Filipino women showed that only 17.9% met the recommended dietary energy requirement; 50.1% met the protein RDA; 12.3% met the iron RDA; and was 16% lower at meeting Vitamin A RDA.

On the other hand, 27% met the recommended dietary intake for energy; less than half met at least 80% of protein requirements; and close to one-fourth of the preschool children met at least 80% of recommended intake for iron. Less than1/10 of the preschool children met at least 80% of recommended intake for zinc; about 17% met the recommended intake for calcium and more than 18% met the recommended intake for Vitamin A. The Philippines Food Consumption Survey (2008) showed that only 17.8% of Filipino preschoolers met the recommended dietary energy intake; 48.3% met the protein RDA; 25.2% met the iron RDA and 26% met the vitamin A RDA.

CONCLUSIONS

Cambodia

The study in both season in dry and rainy season concluded that Cambodia's natural resources provides a foundation for food security, income and employment for their livelihood. Most of the rural people rely on rice cultivation, harvesting of fish and OAAs. Rice and fish are the traditional staple foods playing an important role in the diets of women and children. Rice is the main source of energy and fish is the main source of animal protein. Fish is the major contributor of key micronutrients such as iron, zinc, calcium and vitamin A in women and children in both raining and dry season, but is a lower contributor in the dry season compared with rainy season. Nutritional status of the rural poor women and children was low in both season but in dry season even low compared to rainy season. The low intake of micronutrients in comparison to the recommended daily intake in dry season put them in the risk of micronutrient deficiencies. Fish is a nutritionally important animal food source contributing to the daily diets of the women and children in poor rural households.

Vietnam

The diets of sampled women were basically rice-vegetable-fish combination, in which 72% comes from plant source, 24% comes from animal source, and 3.7% comes from food group such as condiments and spices whereas it was different to preschool children diet. Diet of sampled pre-school children sharing from animal source accounted for 59.2%, plant source contributed 38.9%, and 1.9% came from condiments and spices. The overall mean one day per capita food consumption of women and preschool children was 750 g/p/day (1411 Kcal) and 638 g/p/day (764 Kcal), in raw as purchased form, respectively. Rice was highly consumed in both women and preschool children at 230g/p/day and 153 g/p/day, accounting for 30.7% and 22.5%, respectively. Milk and milk products were the top intake of children, 259.9 g (38%). Vegetables and fish were the second top food group commonly consumed by sample women at around 16.3 and 16.9%, respectively, of the total food intake per capita per day. Twenty-one (21) and 19 of fish species including OAAs (crab, shrimp, etc.) and its products consumed by sample women and preschool children, respectively, in which snakehead (*C. striata*) and red tilapia (*Oreochromis* sp.) were the 1st and 2nd commonly consumed. Fish and OAAs was the second largest food group consumed by women, estimated at 127 g/p/day, sharing nearly at

16.9% to total diet intake. In diet of women, fish and fish products are major contributors of protein at 41.71%, fats at 22.15 %, Iron at 9.53%, calcium at 40.7% and vitamin A at 25.39%, while for preschool children, milk and milk products played an important role for nutrient intake.

QUANTIFIED ANTICIPATED BENEFITS

This investigation has provided recommendations for better nutrition in women and children in Cambodia. Two Master's students were involved in this investigation (two females). Three undergraduate students were also supported for their dissertations (3 females). Six IFReDI staff and Cantho University were involved in the survey, such as the project preparation, data collection and training activities. Four hundred and fifty women and 450 children benefited from the project by improving nutritional status through the proposed nutritional recommendations for adaption options and strategies in Cambodia and Vietnam. One thousand IFReDI/FiA and Cantho University staff, scientists, researchers, government officers and managers, and NGOs understand the nutrition contexts especially in women and children in Cambodia and Vietnam through in series of consultation meeting/workshop and sharing research result findings such as policy brief and technical report. One thousand and two hundred (1200) fact sheets and policy briefs were delivered to IFReDI/FiA staff, scientists, researchers, government officers, NGOs, and women which are direct and indirect benefits from the projects.

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TABLES AND FIGURES

Table 1. Percent distribution of the women's mean daily per capita food consumption across the provinces

Food Group	All Sample Women		Stung Treng Province		Kampong Thom Province		Prey Veng Province	
	Consumption Raw AP (g/day)	% of Total	Consumption Raw AP (g/day)	% of Total	Consumption Raw AP (g/day)	% of Total	Consumption Raw AP (g/day)	% of
Energy-Giving Foods								
Cereals & products	359.0	42.9	356.1	43.2	355.2	42.3	365.9	43.4
Sugars & syrup	5.0	0.6	2.8	0.3	5.9	0.7	6.3	0.7
Starchy roots & tubers	4.0	0.5	4.6	0.6	3.8	0.4	3.7	0.4
Fats and Oils	4.8	0.6	3.1	0.4	4.7	0.6	6.5	0.8
Body-Building Foods								
Fish & OAAs	137.4	16.4	139.1	16.9	140.5	16.7	132.7	15.7
Meat & poultry	40.7	4.9	49.0	5.9	27.0	3.2	46.0	5.5
Eggs	8.8	1.1	4.5	0.5	8.4	1.0	13.5	1.6
Milk & products	4.4	0.5	2.0	0.2	6.1	0.7	5.0	0.6
Legumes, nuts & seed	4.2	0.5	3.6	0.4	5.0	0.6	3.8	0.5
Regulating Foods								
Vegetables	148.9	17.8	147.3	17.9	150.5	17.9	149.0	17.7
Fruits	54.5	6.5	51.5	6.2	70.3	8.4	41.8	5.0
Miscellaneous								
Beverages	53.0	6.3	49.7	6.0	50.6	6.0	58.7	7.0
Condiments & Spices	10.9	1.3	9.9	1.2	11.8	1.4	11.0	1.3
All Foods	835.6	100.0	823.2	100.0	839.8	100.0	843.9	100.0

Table 2. Percent Distribution of the preschool-age children's mean daily per capita food consumption across the provinces

Food Group	All Sample Children		Stung Treng Province		Kampong Thom Province		Prey Veng Province	
	Consumption Raw AP (g/day)	% of Total	Consumption Raw AP (g/day)	% of Total	Consumption Raw AP (g/day)	% of Total	Consumption Raw AP (g/day)	% of Total
Energy-Giving Foods								
Cereals & products	229.5	50.5	245.5	53.8	215.6	48.5	227.5	49.2
Sugars & syrup	5.9	1.3	4.2	0.9	6.4	1.4	7.1	1.5
Starchy roots & tubers	1.8	0.4	0.1	0.0	0.0	0.0	5.3	1.2
Fats and Oils	0.9	0.2	0.9	0.2	0.6	0.1	1.1	0.2
Body-Building Foods								
Fish & OAAs	49.1	10.8	49.3	10.8	47.2	10.6	50.7	11.0
Meat & poultry	9.9	2.2	10.6	2.3	7.1	1.6	12.1	2.6
Eggs	7.5	1.7	7.2	1.6	6.3	1.4	9.1	2.0
Milk & products	45.2	9.9	39.1	8.6	47.5	10.7	48.9	10.6
Legumes, nuts & seed	0.2	0.0	0.1	0.0	0.5	0.1	0.1	0.0
Regulating Foods								
Vegetables	19.5	4.3	16.5	3.6	20.4	4.6	21.6	4.7
Fruits	28.6	6.3	28.5	6.2	26.3	5.9	30.9	6.7
Miscellaneous								
Beverages	53.4	11.7	51.6	11.3	63.6	14.3	44.9	9.7
Condiments & Spices	2.8	0.6	2.6	0.6	3.1	0.7	2.6	0.6
All Foods	454.2	100.0	456.1	100.0	444.6	100.0	461.9	100.0

Table 3. Ten commonly consumed fish species and percent distribution of the women's mean daily per capita fish consumption

No.	Khmer Name	Scientific Name	Mean	%
1	Trye Riel	*Cirrhinus sp.*	30	21.7
2	Trye Ros	*Channa striata*	18.2	13.2
3	Trey Chrakeng	*Puntioplites proctozystron*	14	10.1
4	Trey Changwa phlieng	*Esomus longimanus*	11.6	8.4
5	Trey Slek Reussey	*Parachela siamensis*	9.2	6.7
6	Trey Kamphlienh Sre	*Trichopodus trichopterus*	8.5	6.2
7	Trey Chhviet	*Pangasius macronema*	6.5	4.7
8	Trey Srakar kdam	*Cyclocheilichthys apogon*	5.2	3.8
9	Trey Kranh	*Anabas tastudineus*	4.3	3.1
10	Trey Ta orn	*Ompok hypophthalmus*	4.2	3.0
11	Other species		25.7	19.2

Table 4. Ten commonly consumed fish and percent distribution of the preschool-age children's mean daily per capita fish consumption

No.	Khmer Name		Scientific Name	Mean (g)	%
1	Trey Riel	ត្រីរៀល	*Cirrhinus sp.*	11.9	24.1
2	Trey Ros	ត្រីរ៉ស់	*Channa striata*	9.2	18.6
3	Trey Changwa phlieng	ត្រីចង្វាភ្លៀង	*Esomus longimanus*	4.5	9.1
4	Trey Ta orn	ាអោន	*Ompok hypophthalmus*	3.9	7.9
5	Trey Chhlang	ត្រីឆ្លាំង	*Hemibagrus sp.(cf.nemarus)*	2.7	5.5
6	Trey Andeng	្តដែរឹង	*Clarias batrachus*	2.4	4.9
7	Trey Slek Reussey	ត្រីស្លឹកឫស្សី	*Parachela siamensis*	1.2	2.4
8	Trey Chongva	ត្រីចង្វា	*Rasbora sp.*	1.2	2.4
9	Trey Chrakeng	កែង	*Puntioplites proctozystron*	1.1	2.2
10	Trey Kahe krorhorm	រីក្រម៉ម	*Barbus schwanenfeldii*	1.0	2.0
11	Other species			10.0	*20.9*

Table 5. Mean daily intake and percent adequacy of energy and nutrient intake of Cambodian women surveyed in this study.

Energy and Nutrient	All Women	Stung Treng	Kampong Thom	Prey Veng
Energy(Kcal)	1917.5	1984.2	1780.7	1987.6
Meeting 100% of Energy Intake (%)	48.5	50.8	41.3	53.5
Protein(g)	63.8	65.8	60.8	64.7
Meeting 80% of Protein Intake (%)	68.9	73.2	64.8	68.7
Iron(mg)	12.6	11.4	13.7	12.6
Meeting 80% of Iron Intake (%)	10.3	12.7	4.9	13.4
Zinc(mg)	3.7	3.0	4.1	3.9
Meeting 80% of Zinc Intake (%)	35.3	27.3	45.2	33.4
Calcium(g)	528.8	467.1	528.1	591.2
Meeting 80% of Calcium Intake (%)	23.0	20.5	25.5	22.9
Vitamin A(mcg RE)	445.5	518.9	431.0	386.5
Meeting 80% of Vitim A Intake (%)	27.2	23.4	28.5	29.6
Carbohydrate(g)	345.3	338.5	342.8	354.6
Fats(g)	34.4	32.7	28.4	42.0

Table 6. Mean daily consumption and percent adequacy of energy and nutrient intake of the Cambodian preschool-children surveyed in this study

Energy and Nutrient	All Children	Stung Treng	Kampong Thom	Prey Veng
Energy(Kcal)	780.8	757.7	805.2	779.5
Meeting 100% of Energy Intake (%)	27.0	21.7	27.7	31.6
Protein(g)	26.5	26.1	25.6	27.8
Meeting 80% of Protein Intake (%)	48.2	45.6	47.1	52.1
Iron(mg)	4.7	4.7	4.6	4.8
Meeting 80% of Iron Intake (%)	21.6	14.9	22.2	27.6
Zinc(mg)	1.5	1.2	1.6	1.8
Meeting 80% of Zinc Intake (%)	7.0	2.8	5.5	12.6
Calcium(g)	254.3	202.7	279.1	281.0
Meeting 80% of Calcium Intake (%)	18.4	14.9	17.5	22.9
Vitamin A(mcg RE)	225.0	227.9	259.8	187.2
Meeting 80% of Vitim A Intake (%)	17.0	13.0	22.2	15.8
Carbohydrate(g)	123.2	116.7	123.6	129.2
Fats(g)	13.8	12.8	12.7	16.0

Table 7. Percentage contribution of particular food groups to total energy and nutrient intakes of Cambodian women surveyed in this study.

Food Group (%)	Energy	Protein	Fats	Cars	Iron	Zinc	Calcium	Vit A
Cereals & products	59.2	30.0	14.4	74.9	43.1	16.8	21.4	7.6
Starchy roots & tubers	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.0
Legumes, nuts& seed	0.1	0.2	0.5	0.0	0.3	0.3	0.0	0.0
Vegetables	2.0	4.0	1.2	6.2	14.1	15.1	15.7	25.9

Food Group (%)	Energy	Protein	Fats	Cars	Iron	Zinc	Calcium	Vit A
Fruits	1.4	0.8	0.4	1.7	3.1	2.5	1.4	29.1
Meat & poultry	3.5	10.7	16.5	0.0	7.2	32.4	3.7	0.3
Fish & OAAs	9.4	47.5	27.6	12.1	25.8	27.0	38.5	24.2
Eggs	0.6	1.4	7.0	0.0	1.7	1.1	4.2	3.2
Milk & products	0.1	0.1	4.6	0.1	0.1	0.2	3.6	0.1
Fats & Oils	1.1	0.0	13.4	0.0	0.0	0.1	0.1	0.1
Sugars & syrup	0.2	0.1	0.3	0.2	0.0	0.0	0.4	0.1
Condiments and Spices	0.3	1.1	0.5	0.1	4.3	1.0	6.7	2.8
Beverages	19.0	1.2	10.6	1.5	0.3	0.6	1.3	0.8

Table 8. Percentage contribution of particular food groups to total energy and nutrient intakes for Cambodian preschool children surveyed in this study.

Food Group (%)	Energy	Protein	Fats	Cars	Iron	Zinc	Calcium	Vit A
Cereals	53.4	34.6	21.5	73.5	44.5	4.4	12.1	2.1
Starchy roots and tubers	0.3	0.1	0.1	0.5	0.4	0.3	0.2	0.0
Legumes, nuts and seed	0.1	0.2	0.7	0.0	0.4	0.4	0.0	0.0
Vegetables	0.8	1.5	0.5	5.7	6.6	6.9	5.4	11.7
Fruits	3.5	1.3	1.3	4.8	7.3	5.9	2.1	38.1
Meat & poultry	2.4	7.6	9.3	0.1	5.3	19.5	0.4	0.7
Fish & OAAs	11.3	40.9	28.4	0.9	14.6	18.2	34.9	16.6
Eggs	1.9	3.8	9.6	0.2	5.6	3.6	2.3	12.1
Milk and products	7.2	7.4	12.0	7.4	11.5	39.7	36.3	16.7
Fats and Oils	1.1	0.0	7.9	0.0	0.0	0.0	0.0	0.0
Sugars and syrup	2.0	0.6	3.2	2.1	0.2	0.0	1.8	0.0
Condiments and Spices	0.3	0.8	0.4	0.1	3.1	1.0	2.9	1.2
Beverages	8.6	1.2	5.2	4.7	0.6	0.1	1.5	0.8

Table 9. Percentage contribution of fish to total animal energy and nutrient intakes for Cambodian women surveyed in this study.

Animal Food Source (%)	Energy	Protein	Fat	Cars	Iron	Zinc	Calcium	Vit A
Meat & poultry	24.2	17.0	30.5	0.3	19.6	50.6	7.6	0.9
Fish & OAAs	66.0	75.4	51.1	94.0	70.5	42.2	78.5	82.7
Eggs	4.4	2.2	12.9	0.3	4.5	1.7	8.5	11.0

Table 10. Percentage contribution of fish to total animal energy and nutrient intakes for Cambodian preschool children surveyed in this study.

Animal Food Source (%)	Energy	Protein	Fat	Cars	Iron	Zinc	Calcium	Vit A
Meat & poultry	14.4	13.6	18.3	10.6	19.4	43.8	1.0	2.2
Fish & OAAs	67.0	72.5	55.8	87.1	53.1	41.0	86.2	52.4
Eggs	11.5	6.8	18.8	4.2	20.4	8.1	5.7	38.2

Table 11. Percent Distribution of the women's mean one-day per capita food consumption in An Giang province, Vietnam

Food Group	All Sample Women	
	Consumption Raw AP (g/day)	% of Total
Energy-Giving Foods		
Rice (mean food)	230.0	30.7
Cereals & products	62.9	8.4
Sugars & syrup	10.5	1.4
Starchy roots & tubers	13.4	1.8
Fats and Oils	1.9	0.3
Body-Building Foods		
Fish & OAAs	127.0	16. 9
Meat & poultry	83.0	11.1
Eggs	18.9	2.5
Milk & products	9.8	1.3
Legumes, nuts & seed	0	0.0
Other Aquatic products	8.8	1.2
Regulating Foods		
Vegetables	122.0	16.5
Fruits	52.2	7.0
Miscellaneous		
Beverages	2.7	0.4
Condiments & Spices	7.3	1.0
All Foods	**750.3**	**100**

Table 12. Percent Distribution of the preschool-age children's mean one-day per capita food consumption across the provinces in Vietnam

Food Group	All Sample Children	
	Consumption Raw AP (g/day)	% of Total
Energy-Giving Foods		
Rice	153.5	22.5
Cereals& products	65. 4	9.6
Sugars &syrup	4.1	0.6
Starchy roots &tubers	4.4	0. 6
Fats and Oils	0.7	0.1
Body-Building Foods		
Fish & OAAs	55.4	8.1
Meat & poultry	62.2	9.1
Eggs	15	2.2
Milk &products	259.9	38.0

Food Group	All Sample Children	
	Consumption Raw AP (g/day)	% of Total
Other aquatic products	11.7	1.7
Regulating Foods		
Vegetables	20.6	3.0
Fruits	22.0	3. 2
Miscellaneous		
Beverages	6.5	1.0
Condiments &Spices	1.7	0.2
All Foods	683	100

Table 13. First 10 commonly consumed fish and percent distribution of the women's mean one day per capita fish consumption in Vietnam

No.	Vietnamese name	Common name	Scientific name	Mean	%
1	Cá lóc	Snakehead	*Channa striata*	39.1	28.8
2	Cá điêu hồng	Red tilapia	*Oreochromis* sp.	26.2	19.3
3	Cá rô đồng	Climbing perch	*Anabas tastudineus*	5.4	4.0
4	Cá tra	Striped catfish	*Pangasianodon hypophthalmus*	17.6	13.0
5	Lươn	Swam eel	*Monopterus albus*	8.0	5.9
6	Cá trê	Clarias catfish	*Clarius* spp.	4.0	2.9
7	Mè vinh, he, chép	Siver barb, common carp	*Barbonymus gonionotus, Barbonymus schwanenfeldii, Ciprinus carpio*	8.1	6.0
8	Ếch	Frog	*Hoplobatrachus rugulosus*	66.2	44.6
9	Cá lăng	Bagridae fish	*Hemibagrus wyckioides*	6.5	4.8
10	Tôm, tép, cua, ghẹ	Shrimp, prawn, crab, blue swimming crab	Penaeidae shrimp, *Macrobrachium resenbergii,* decapod crustaceans	4.2	3.1
11	Other species			10.4	7.7
	Total			**135.7**	**100**

Table 14. First 10 commonly consumed fish and percent distribution of the preschool-age children's mean one day per capita fish consumption in Vietnam

No	Vietnamese name	Common name	Scientific name	Mean	%
1	Cá lóc	Snakehead	*Channa striata*	28.5	2.5
2	Cá điêu hồng	Red tilapia	*Oreochromis* sp.	10.2	5.2
3	Cá rô đồng	Climbing perch	*Anabas tastudineus*	2.0	.0
4	Cá tra	Striped catfish	*Pangasianodon hypophthalmus*	7.4	1.0
5	Lươn	Swam eel	*Monopterus albus*	8.0	1.9
6	Cá trê	Clarias catfish	*Clarius spp.*	0.1	0.1
7	Mè vinh, he, chép	Siver barb, common carp	*Barbonymus gonionotus, Barbonymus schwanenfeldii, Ciprinus carpio*	0.5	0.7
8	Ếch	Frog	*Hoplobatrachus rugulosus*	3.5	5.2
9	Cá lăng	Bagridae fish	*Hemibagrus wyckioides*	2.2	3.3
10	Tôm, tép, cua, ghẹ	Shrimp, prawn, crab, blue swimming crab	Penaeidae shrimp, *Macrobrachium resenbergii,* decapod crustaceans	2.5	3.7
11	Cá thát lát	Bronze featherback	*Notopterus notopterus*	2.3	1.2
12	Other species			1.4	2.1
	Total			**67.1**	**100**

Table 15. Percentage contribution of particular food groups to the women's total energy and nutrient intakes in Vietnam

Food group (%)	Energy	Protein	Fats	Carbohy drate	Iron	Calcium	Zinc	Vit A
Rice (mean food)	56.17	24.62	10.67	70.11	39.71	18.35	15.99	7.22
Cereals & products	6.32	1.11	0.00	1.11	2.72	2.88	3.25	0.00
Sugars & syrup	2.56	0.71	0.28	3.69	1.29	1.27	0.57	0.00
Fats and Oils	1.12	0.00	12.39	0.26	7.70	0.23	1.32	0.00
Starchy roots & tubers	1.57	0.80	0.93	4.87	1.30	0.11	0.35	0.33
Vegetables	3.63	1.33	1.08	4.41	7.30	3.63	6.37	26.19
Fruits	3.22	1.41	0.95	0.04	8.55	4.28	5.69	28.12
Meat & poultry	10.06	19.32	29.43	0.11	16.41	5.89	28.77	0.96
Eggs	2.88	6.44	12.43	0.14	1.65	6.87	4.14	6.71
Milk & products	0.38	0.30	7.03	0.36	0.07	10.14	0.44	0.41
Fish & OAAs	10.27	41.71	22.15	13.24	9.53	40.70	29.46	25.39
Aquatic product others	0.60	0.27	1.04	0.94	1.83	1.40	0.00	0.33
Condiments & Spices	1.16	1.93	1.54	0.74	0.02	3.96	3.63	4.10
Beverages	0.06	0.03	0.03	0.00	1.96	0.27	0.00	0.20
All Foods	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 16. Percentage contribution of particular food groups to the preschool-age chidren's total energy and nutrient intakes in Vietnam

Food Group (%)	Energy	Protein	Fats	Carbohy drate	Iron	Calcium	Zinc	Vit A
Rice (mean food)	28.8	9.30	10.00	84.50	69.40	15.60	46.70	7.01
Cereals & products	12.4	0.40	1.80	0.40	2.60	0.70	1.40	0.00
Sugars & syrup	1.9	0.00	0.00	2.20	0.03	0.00	0.00	0.00
Fats and Oils	0.3	0.00	4.50	0.00	0.00	0.00	0.00	0.00
Starchy roots & tubers	0.6	0.05	0.02	0.50	0.60	0.10	0.20	0.00
Vegetables	0.9	0.20	0.08	0.70	2.10	1.40	0.70	0.00
Fruits	1.7	0.10	0.30	1.60	1.90	0.80	1.00	0.00
Meat & poultry	12.4	6.70	20.80	0.00	7.20	0.90	23.70	3.20
Eggs	3.5	1.10	8.70	0.10	5.00	2.00	1.90	19.20
Milk & products	27.0	74.40	46.30	9.10	3.90	65.10	21.80	64.20
Fish & OAAs	7.7	6.30	6.70	0.08	4.90	10.50	0.70	5.80
Aquatic product others	1.6	0.90	0.30	0.10	1.90	1.50	1.60	0.60
Condiments & Spices	1.0	0.50	0.20	0.60	0.60	1.20	0.06	0.00
Beverages	0.2	0.01	0.20	0.20	0.10	0.10	0.04	0.00

Table 17. Percentage contribution of fish to the women's total animal energy and nutrient intakes in Vietnam

Animal Food Source (%)	Energy	Protein	Fats	Carbohyd rate	Iron	Calcium	Zinc	Vit A
Meat & poultry	43.33	28.64	45.98	0.82	59.50	11.02	46.13	2.91
Fish & OAAs	44.25	61.82	34.60	98.1	34.53	76.13	47.24	76.80
Eggs	12.42	9.55	19.42	1.04	5.97	12.85	6.63	20.29
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 18. Percentage contribution of fish to the preschool-age children's total animal energy and nutrient intakes

Animal Food Source (%)	Energy	Protein	Fats	Carbohydrate	Iron	Calcium	Zinc	Vit A
Meat & poultry	52.38	47.52	57.46	0.55	42.11	6.72	90.11	11.35
Fish & OAAs	32.72	44.68	18.51	44.20	28.65	78.36	2.66	20.57
Eggs	14.89	7.80	24.03	55.25	29.24	14.93	7.22	68.09
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

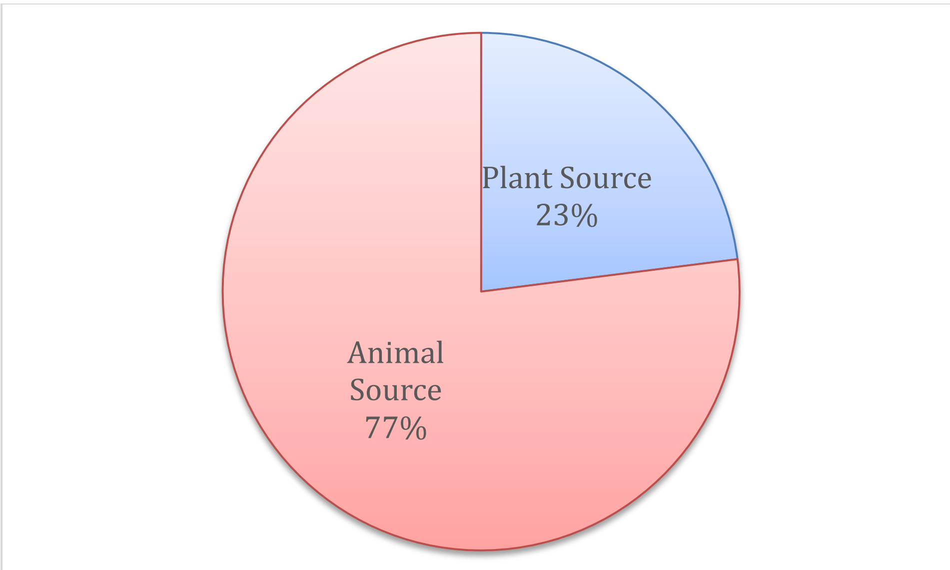

Figure 1. Percent contribution of food sources of mean daily per capita food consumption for Cambodian women surveyed in this study.

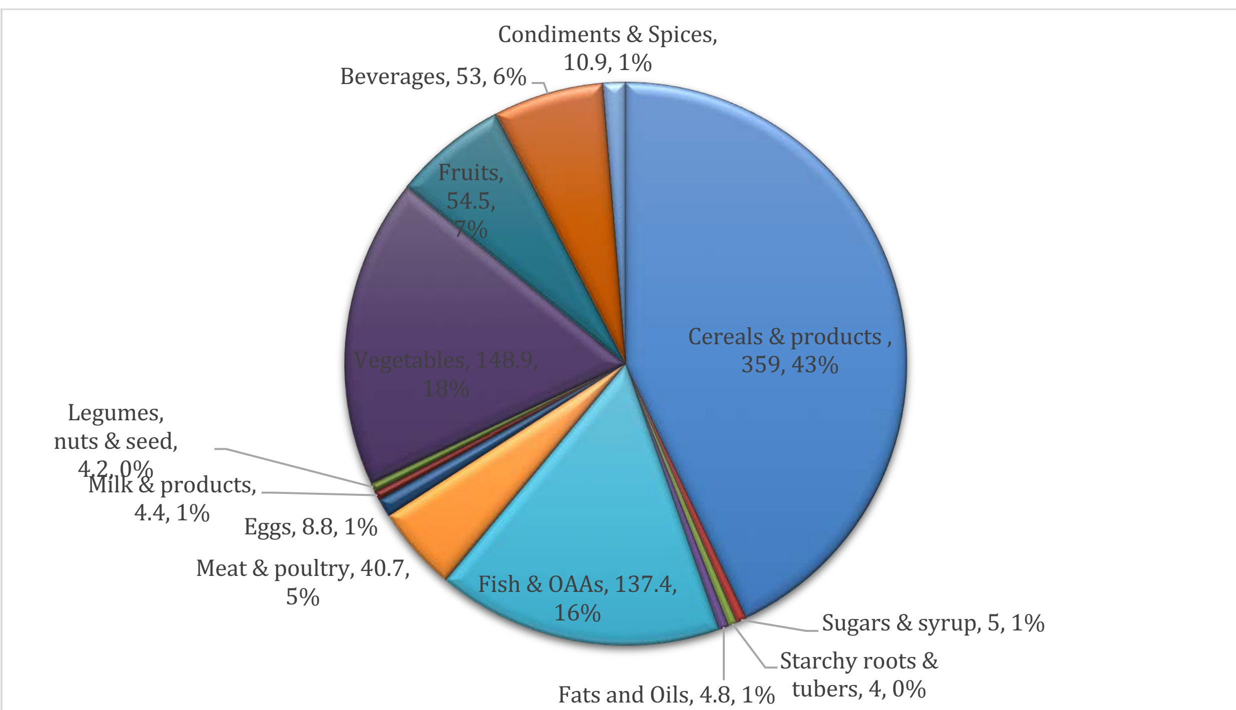

Figure 2. Percent distribution of mean daily per capita food consumption by particular food group for Cambodian women surveyed in this study.

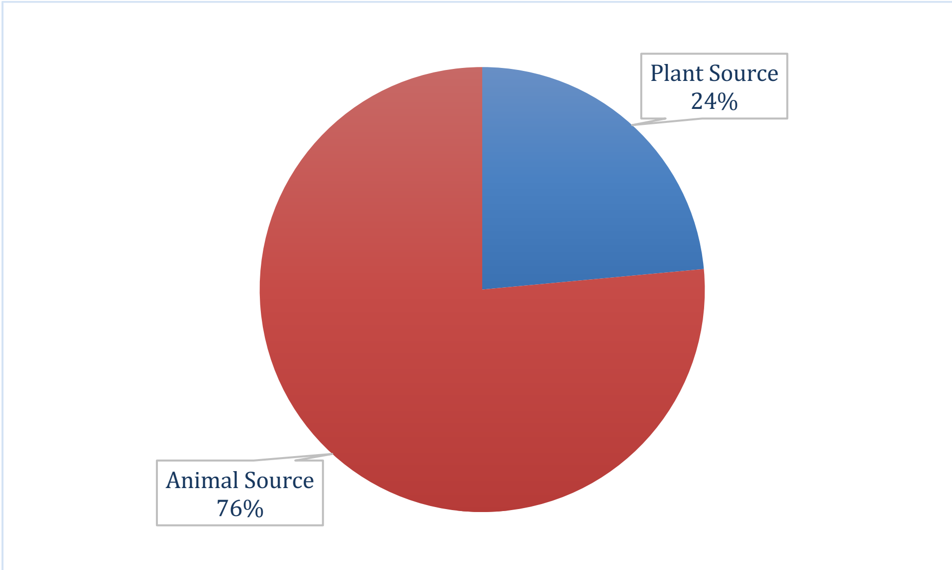

Figure 3. Percent contribution of food sources to mean daily per capita food consumption by Cambodian preschool children surveyed in this study.

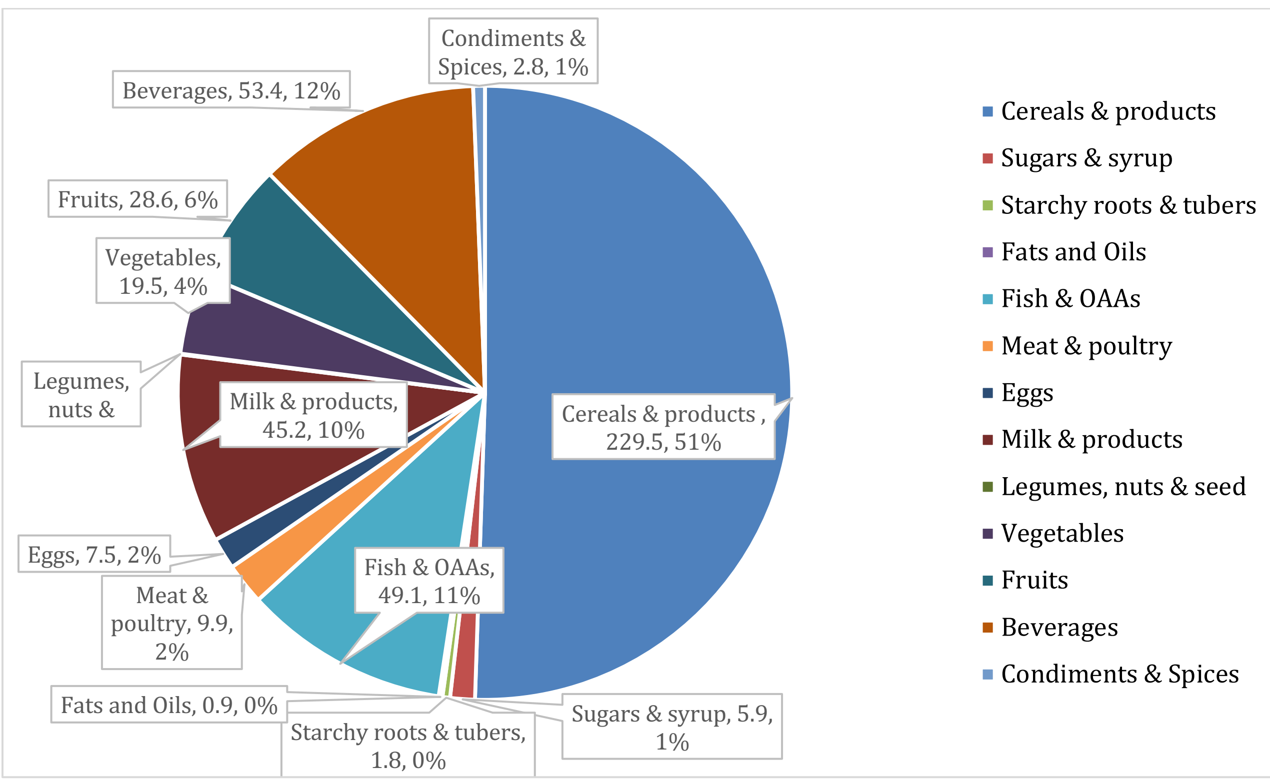

Figure 4. Percent distribution of the mean daily per capita food consumption by particular food group for Cambodian preschool children surveyed in this study.

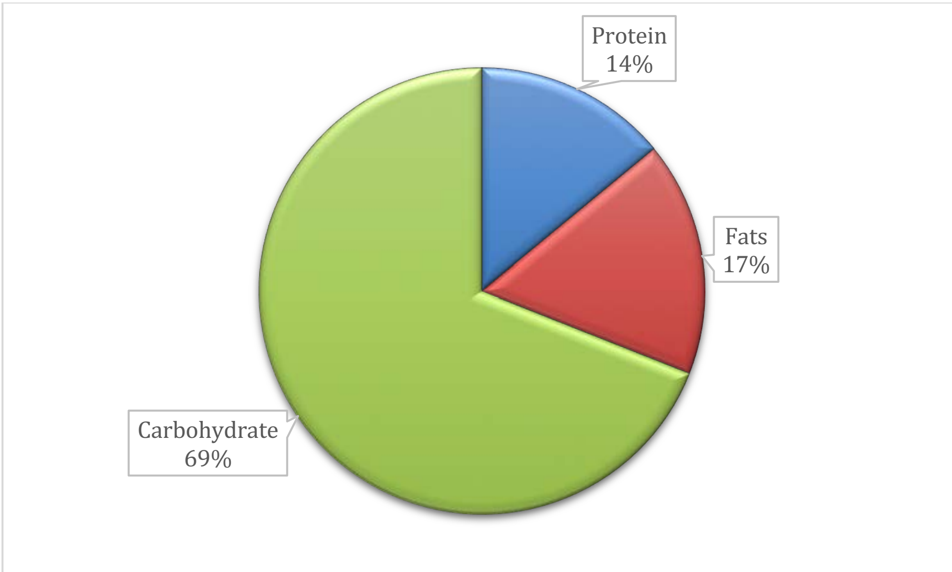

Figure 5. Percent contribution of carbohydrate, fats and protein to total dietary energy for Cambodian women surveyed in this study.

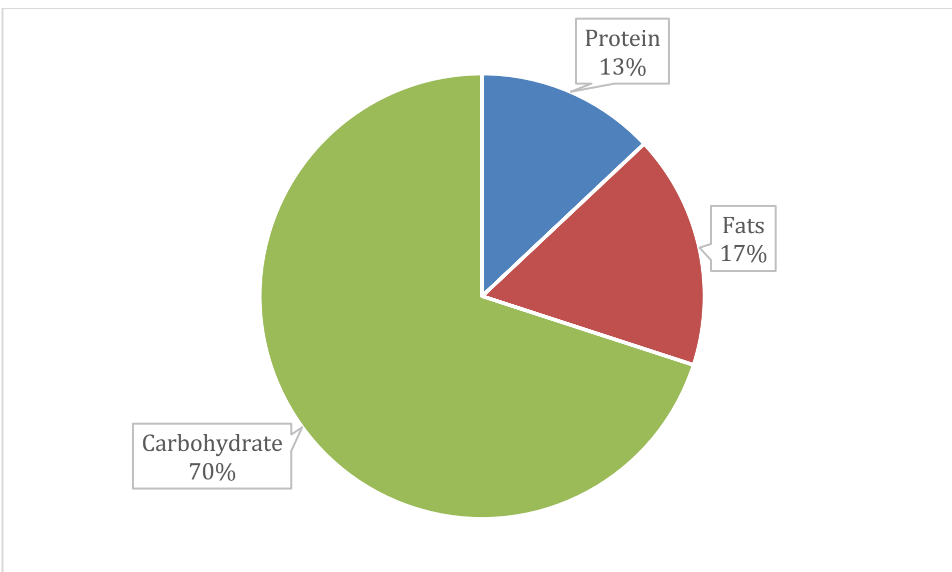

Figure 6. Percent contribution of carbohydrate, fats and protein to total dietary energy for Cambodian preschool children surveyed in this study.

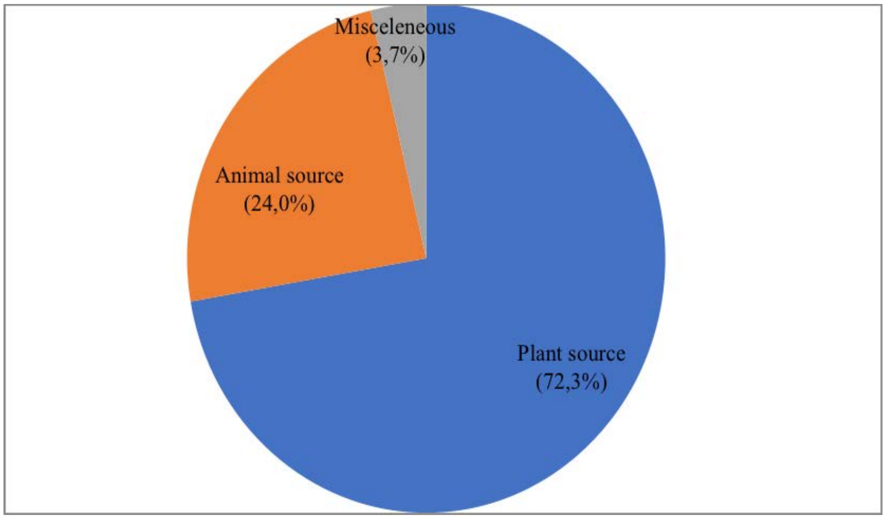

Figure 7. Percent contribution of food sources of the women's mean one day capita food consumption in Vietnam

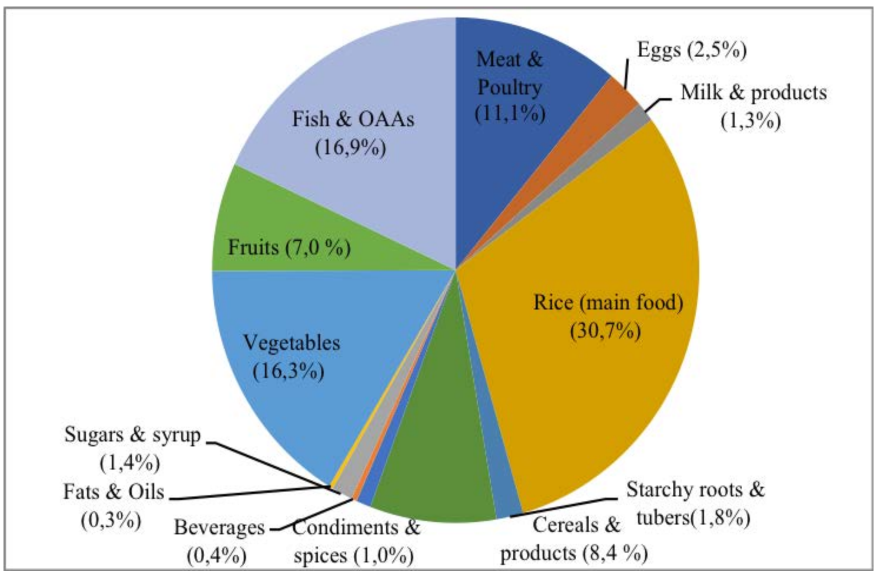

Figure 8. Percent Distribution of the women's mean one-day per capita food consumption by particular food group.

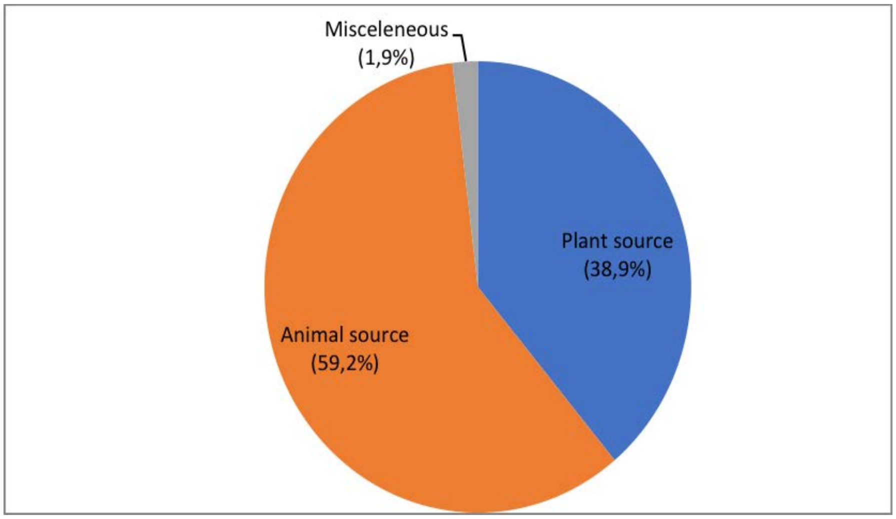

Figure 9. Percent contribution of food sources of the preschool-age children's mean one day capita food consumption in Vietnam

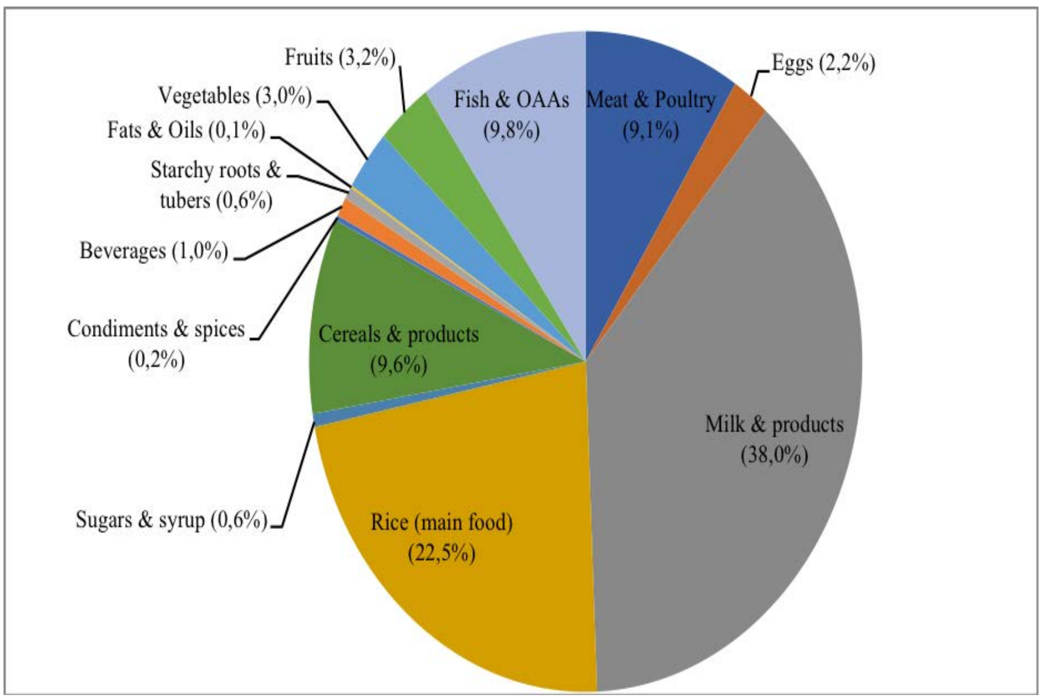

Figure 10. Percent Distribution of the preschool-age children's mean one-day per capita food consumption by particular food group in Vietnam.

ENHANCING FOOD SECURITY AND HOUSEHOLD NUTRITION OF WOMEN AND CHILDREN THROUGH AQUACULTURE AND CAPTURE FISHERIES IN CAMBODIA AND VIETNAM IN THE DRY SEASON- PART II

Food Safety, Post-Harvest, and Value-Added Product Management/Study/16FSV01UC

Touch Bunthang[1], So Nam[1], Cheng Phen[1], Nei Somalay[2], Chan Channsreyrath[2], Tey Boramey[2], Hillary Egna[3], and Robert Pomeroy[4]

[1]Inland Fisheries Research and Development Institute, Fisheries Administration, Phnom Penh, Cambodia;
[2]Royal University of Agriculture, Phnom Phen
[3]College of Agricultural Science, Oregon State University, Corvallis, OR, USA
[4]Department of Agricultural and Resource Economics/CT Sea Grant, University of Connecticut,CT, USA

ABSTRACT

In Cambodia where malnutrition remains a key development challenge, fish is an exceptional and cheaper animal-source food in the diets and livelihoods of millions. Knowledge of nutrient composition of fish from Cambodia is poor and the pre-existing data on the nutrient components of fish do not reflect the large diversity available and have focused on only a few select species and nutrients. The present study aimed to analyze the nutrient contents of 8 selected fresh and two processed fish species commonly consumed by women and preschool children in Cambodia with a specific focus on calcium, iron, phosphorus, protein, fat, carbohydrate, energy, ash and moisture as a supplementary data to our previous nutrient evaluation on other commonly-consumed fresh and processed fish, and other aquatic animal species. When comparing species, the composition of nutrients of public health significance was diverse. In fresh fish, iron ranged from 1.11 to 5.11 mg/100g, phosphorous (116.33 to 512.5 mg/100g) and calcium (126.84 to 534.48 mg/100g). Several species were rich in calcium, particularly *Esomus longimanus* (355.69 mg/100g), *Cyclocheilichthys apogon* (534.48 mg/100g), *Parachela siamensis* (423.65 mg/100g) and *Barbus schwanenfeldii* (512.44 mg/100g), largely when small fish are eaten whole with bones included in the edible parts. In processed fish, iron ranged from 6.09 to 6.96 mg/100g, phosphorous (893.05 to 1,280.62 mg/100g) and calcium (471.5 to 2,488.25 mg/100g), as well as other tested components include protein, fat, carbohydrate, energy and ash in general showing greater values than in fresh fish, except moisture having lower values due to smoked-drying process reduced water in processed fish. These data illustrate the diversity in nutrient composition of fish species and in particular the rich nutrient components of small species, which should guide policy and programs to improve food and nutrition security in Cambodia, particularly for vulnerable groups, women and preschool children.
Keywords: Small fish species, nutrient density, proximate component, women, children, Cambodia

INTRODUCTION

The food and nutritional consumption survey in dry season in Cambodia in women and preschool children within Investigation 5 found that women commonly consume 40 species of fish and OAA and preschoolers commonly eat 35 species of fish and OAAs (first FIR for Investigation 5). Eight selected fresh and two processed fish species commonly consumed by women and preschool children in Cambodia with a specific focus on calcium, iron, phosphorus, protein, fat, carbohydrate, energy, ash and moisture as a supplementary data to our previous nutrient evaluation on other commonly-consumed fresh and processed fish, and other aquatic animal species (table 1).

In Cambodia, fish, fish products and other aquatic animal (OAAs) species are an irreplaceable and cheaper animal-source food in the diets and livelihoods of millions, both in terms of quantity - accounting for roughly 12% of the total energy intake, 28% of total fat intake, 37% of iron consumed, 37% of total protein intake and 76% of animal protein intake at 173 g per person per day that is 63 kg per person per year - and very high frequency of consumption, far exceeding that of any other animal-source food (Mogensen, 2001; Roos et al., 2003; FAO and WHO, 2004; Hortle, 2007; Chamnan et al., 2009; So and Touch, 2011; IFReDI, 2013; Baran et al., 2014; FAO, 2014; Vilain and Baran, 2016). Thus, fish plays an extraordinarily important role in Cambodia's national food and nutrition security (Chamnan et al., 2009; Vilain and Baran, 2016). The country possesses diverse and abundant aquatic resources with more than 490 freshwater fish species and features the fifth in inland fisheries productivities after China, India, Bangladesh and Myanmar with an annual average of 400,000 tones (Baran et al., 2014; Vilain and Baran, 2016). Despite improvement in some food and nutrition security indicators, malnutrition, largely caused by inadequate micronutrient intake, remains widespread with 6.7% of population suffering from severely underweight, 39.9% moderate and severe stunting and 10.9% wasting (NIS et al., 2011; So and Touch, 2011; Vilain and Baran, 2016). Malnutrition kills more than 6,400 children per year in Cambodia (UNICEF Australia, 2014) and school-aged children are the most energy deficient (IFReDI, 2013). Eighty-nine percent (89%) of rural people achieve the recommended intake of protein, however only 51% of the population meets the adequate energy intake, 25% reach recommended levels of energy and 19% meet the recommended levels of iron. In general, women and children under five years are the vulnerable groups receiving more sensitive to deficiencies (SCN, 2004; Roos et al., 2007c; Vilain and Baran, 2016). Despite the clear importance of fish in Cambodian diets and livelihoods, existing composition data do not reflect the great diversity of species available for consumption and have simply focused on a few select species and nutrients rather than all-inclusive nutrient profiles. The newly pre-established Food Composition Table for Cambodia is a useful compilation of existing composition data on important foods including numerous fish and fish products; however, the data come from various sources including regional databases with varying sampling and analytical methods, some of which are now several decades old (FAO/INFOODS, 2013).

Despite the recently report on nutrient components of 15 selected fresh and processed fish, and three OAAs species commonly consumed by women and preschool children in Cambodia, the nutrient contents of other species describing in food and nutritional consumption survey (Touch et al., 2016a; 2016b) remains still unexplored. Therefore, the present work aimed to determine the nutrient composition of eight selected fresh and two processed fish species that are commonly consumed by Cambodian women and preschool children with a specific focus on calcium, iron, phosphorus, protein, fat, carbohydrate, energy, ash and moisture, which are of known public health concern and food and nutrition insecurity issues in the country as an additional data to our previous nutrient evaluation on other commonly-consumed fresh and processed fish, and OAAs species (Touch et al., 2016b) as well as to extend the data to incorporate more species diversity.

MATERIALS AND METHODS

Sample collection and preparation

Fresh and processed fish samples were purchased from local public markets in Prey Veng, Kampong Thom and Kandal provinces in Cambodia during the dry season of April to May 2017. The length of each fish species sample was measured to the nearest centimeter upon purchasing at the markets to ensure that the samples had uniformity for the analysis. The identification details of each sample including local or common name, scientific name, length and sample preparation details are shown in Table 1. Depending on the fish species, edible parts may or may not include the bones, head, viscera, scales, tail, fins and other parts according to traditional practice commonly-consumed by women and school children (Table 1). To avoid contamination of samples, non-metal equipment such as plastic cutting boards, buckets and strainers, and stainless steel cutting knives were used to obtain raw edible parts. Fish samples were washed with water after dissecting out the head, viscera, scales, tail, fins, bones and other parts on collection site and before being tightly packed in polyethylene bags and transported in a storage box lined with ice chips and away from direct sunlight, to the Industrial Laboratory Centre of Cambodia (ILCC), Ministry of Industry and Handicraft in Phnom Penh for nutrient and proximate composition analyses. Fish species were ground and homogenized as per raw edible parts prior to analysis and subsamples of the homogenate were taken, with size appropriate for individual analytical tests (70-3,000mg). For several species, the homogenate included bones, and for others, bones, head and other parts were removed prior to homogenization if they are typically discarded as plate waste, as shown in Table 1.

Proximate analysis

Protein, energy, moisture and ash contents of fresh and processed fish samples were analyzed according to the standard analytical methods as per the Association of Official Analytical Chemists (AOAC). Protein was determined using based on the total nitrogen content of the Kjeldahl method (AOAC, 2000), whereas energy, fat, moisture and ash were evaluated following the standard methods of AOAC (2005) and carbohydrate was calculated according to FAO (2003). All analytical measurements were performed in 3 independent experiments, each performed in triplicate and mean value were calculated.

Micronutrient analysis

Calcium and phosphorus were analyzed according to the standard methods of AOAC (2005), whereas iron was spectrophotometrically determined following Tsutomu et al. (2007). All analytical measurements were performed in 3 independent experiments, each performed in triplicate and mean value were calculated.

RESULTS AND DISCUSSION

Proximate composition

The energy, protein, fat, carbohydrate, moisture and ash composition of all fish species are shown in Tables 2, 3a and 3b. The total energy content varied greatly with a range of 90.18 to 197.7 Kcal/100g and 426.12 to 589.65 Kcal/100g in fresh and processed fish, respectively which is related to variation in fat content in the different species (Tables 3a and 3b). Several studies indicated that only 51% of the Cambodian population meets the adequate energy intake whereas 25% reach recommended levels of energy. In general, children and women are more sensitive to deficiencies (SCN, 2004; Roos et al., 2007c; Vilain and Baran, 2016). Fish, fish products and OAAs contribute 12% of the total energy intake (Vilain and Baran, 2016), thus consumption of fish alone is not able to ensure adequate energy intake, however with rice combination will provide an additional average of 1,095 Kcal, i.e. 60% of the total energy intake and when the latter cannot be supplied by rice and fish, consume other starchy food items are necessary (IFReDI, 2013). Results of the total protein content in fish species ranged from 14.53 to 18.82% (fresh) and 38.02 to 50.06% (processed) in our study; these can be assumed to be of high dietary quality, being an animal-source protein (WHO, 2007). When comparing the protein content, the processed fish showed higher value than the fresh fish (Ahmed et al., 2011). Fish and OAAs provide 37% of the total protein intake and 76% of the animal protein intake (49% from freshwater fish and 5% from freshwater aquatic animals, 20% from marine fish and animals and 2% from aquaculture fish) (Chamnan et al., 2009; So and Touch, 2011; IFReDI, 2013; Baran et al., 2014; Vilain and Baran, 2016). Several studies reported 89% of rural Cambodian poor reach the recommended intake of protein (SCN, 2004; Roos et al., 2007c; Vilain and Baran, 2016), which may due to the fact that Cambodians consume very high consumption of fish at 63 kg per person per year in addition to their 35% of the total protein intake from other animal and vegetable-source foods (IFReDI, 2013; Baran et al., 2014; Vilain and Baran, 2016). The fat content ranged from 1.92 to 13.99% and 20.18 to 47.15% in fresh and processed fish, respectively. Fat generally varies much more widely than other nutrient components of fish, and usually reflects differences in the way fat is stored in particular species but may also be affected by processing (e.g. smoked-drying), seasonal or lifecycle variations and the diet or food availability of the species at the time of sampling (Ababouch, 2005; Ahmed et al., 2011). Cambodians consume 19 g of total fat per capita per day (Mogensen, 2001). In Cambodia, fish, fish products and OAAs contribute 28% of the total fat intake (IFReDI, 2013; Vilain and Baran, 2016), whereas fish especially the white and black species, account for 27.81 % of the total fat intake of the household (IFReDI, 2013). Fat composition of fish is unique and of high quality fat compared to other animal food sources (Kawarazuka, 2010), particularly the two essential pre-formed long chain omega-3 polyunsaturated fatty acids (PUFA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) that cannot be found elsewhere (McKeown, 2006; Morris, 2008; FAO, 2010; Kawarazuka and Béné, 2010). Fish, especially fatty fish contains high levels of omega-3 fatty acids and should be consumed at least twice a week to combat deficiencies and meet dietary requirements in EPA and DHA, (Fernandes, 2012). The FAO recommends 250 mg EPA and DHA per day for men and non-pregnant women (FAO, 2010), whereas childbearing women require double their normal intake of DHA from diet to accommodate the needs of the fetus or infant (McKeown, 2006). Moreover, to avoid coronary heart disease, it advised to consume at least 225 g of fish or shellfish per week per person (Vannice and Rasmussen, 2014). This represents two or more servings of fish per week with an average daily intake of 450 to 500 mg EPA and DHA (Vilain and Baran, 2016). Results also showed that water is the main constituent in the edible parts ranged from 66.83% to 76.38% in fresh and 5.31% to 9.76% in processed fish, and as expected was negatively associated with fat and energy content (Tables 3a and 3b) and the content was much lower in processed fish in which water may largely be dehydrated during smoked-drying process (Ahmed et al., 2011). Ash content ranged from 0.78 to 3.47% (fresh) and 6.23 to 8.94% (processed) Table 3a and is positively linked with mineral content, particularly calcium, phosphorus and iron whereas fresh fish has lower ash content, most likely the high ash content of processed fish were due to water loss during processing, i.e. smoked-drying concentrated other components such as minerals (Ahmed et al., 2011). The variation in ash content is likely related to inclusion of bones as edible parts in some species, which would lead to higher ash content (Tables 1 and 3a). The content of carbohydrate was also analyzed for data completeness but it is not associated with significant public health concerns currently, and therefore, its nutritional significance is not discussed here. In this study, carbohydrate content ranged from 1.57 to 8.45% in fresh and 3.29 to 11.07% in processed fish. Carbohydrate is generally detected at very low level (lower than 0.5%). In fact, fish is a good source of all nutrients except for carbohydrate and vitamin C. The fish carbohydrate is present in the striated muscle as glycogen and as part of the chemical constituents of nucleotides (Huss, 1995).

Micronutrient composition

The iron, calcium and phosphorus composition for all species are shown in Tables 2, 3a and 3b. Iron content varied considerably with a range from 1.11 to 5.11 mg/100g in fresh and 6.09 to 6.96 mg/100g in processed fish. These results show a greater range in iron content of all fish species compared to those values indicated in our previously report on other selected fresh and processed fish and OAAs species commonly consumed by Cambodian women and preschool children (Touch et al., 2016b) and the ASEAN Food Consumption Table (2000), however slightly lower values than the global FAO/INFOOD database on fish and shellfish (FAO/INFOODS, 2013). Of interest is that iron content of *Esomus longimanus* (Trey Changwa plieng) showing the lowest value (2.04 mg/100g) compared to other species; this could be explained by the fact that in fish, iron is concentrated in the head and viscera which is compatible with our analysis that viscera was excluded (Kawarazuka and Béné, 2010). This may also be partly attributable to sampling and analytical variability, species and size differences, or may reflect real differences in the accumulation of iron in these species based on differing environmental conditions (Huss, 1995; Roos et al., 2007c). The true nature and magnitude of these differences should be further investigated. Overall, the data presented here indicate that several species may contribute significantly to dietary iron intakes in Cambodia which is of high bioavailability as an animal-source food (FAO and WHO, 2004). In Cambodia, fish contributes 37%

of the iron consumed and sour soup made with the *Esomus longimanus* species can cover as much as 45% of the daily iron requirement for women and children. However, iron absorption is reduced by rice consumption containing phytate – the known inhibitor of the absorption of proteins, iron, zinc, calcium and 70% of pregnant women and 74% of children under five years are affected by iron deficiency. These may also have important policy implications given the public health significance of iron deficiency anemia (IDA) in Cambodia, with prevalence recently estimated at around 5% in preschool children and women (Wieringa et al., 2016), and the well-documented negative effects of deficiency on physical and cognitive and immune system development, pregnancy outcomes, morbidity and mortality (WHO, 2001; Roos et al., 2007a).

Calcium content ranged from 126.84 to 534.48 mg/100g in fresh and 471.5 to 2,488.25 mg/100g in processed fish. These results are within the range of fish and seafood reported elsewhere (Kotchanipha et al., 2012; FAO/INFOODS, 2013) and significantly higher than those values reported by the ASEAN Food Consumption Table (2000). As would be expected, calcium content was much higher in species in which bones are commonly consumed and included in the edible parts (Mogensen, 2001; Roos et al., 2007c; IFReDI, 2013; Vilain and Baran, 2016). Of interest, several species were rich in calcium particularly *Esomus longimanus* (Trey Changwa phlieng, 355.69 mg/100g), *Cyclocheilichthys apogon* (Trey Srorka kdam, 534.48 mg/100g), *Parachela siamensis* (Trey Slek reussey, 423.65 mg/100g) and *Barbus schwanenfeldii* (Trey Kahe krorhorm, 512.44 mg/100g). Calcium deficiency among children results in terrible dentition, whereas it leads to hypertension, preterm birth or fetal death among pregnant women. In Cambodia, fish is the second food item consumed after rice with average consumption of 173 g per person per day, i.e. 63 kg per person per year (Baran et al., 2014; Vilain and Baran, 2016) and the most important dietary source of high calcium bioavailability since dairy products are nearly non-existent and if consumed is very low (Roos et al., 2007c; IFReDI, 2013). Many small fish species fully consumed with bones are a rich source of calcium that balance the limited calcium input through dairy products (Roos et al., 2007a; 2007b; 2007c) and small fish constitute up to 86% of the total calcium intake from fish and OAAs as large bones from big fish are not eaten (Mogensen, 2001; Roos, 2001). In particular, the bioavailability of calcium from bones of whole small fish is as high as that from milk (Hansen et al., 1998; Larsen et al., 2000). The data presented here further support the conclusion that in Cambodia, small fish eaten whole with bones are a major source of highly bioavailable dietary calcium (Larsen et al., 2000; Roos et al., 2007a; 2007b; So and Touch, 2011), however large fish do not contribute much to calcium intake because their bones are discarded (Roos, 2001).

Phosphorus content ranged from 116.33 to 512.5 mg/100g (fresh) and 893.05 to 1,280.62 mg/100g (processed), with higher composition in fish species with bones included in edible parts, and consistent with values reported in FAO/INFOODS (2013). Of interest is that phosphorus content of all fish species is much higher than those reported values from the ASEAN Food Consumption Table (2000) and Kotchanipha et al. (2012). This may possibly be partly attributable to species and size differences, edible parts, or differing biodiversity conditions. Phosphorus, like calcium, is also involved in bone and teeth mineralization (Michaelsen et al., 2009; Soetan et al., 2010). Fish, particularly soft-boned fish eaten whole, is an excellent source of highly available phosphorus (Michaelsen et al., 2009). Malnourished children are frequently suffering from hypophosphatemia (low phosphate levels in the body), which can lead to rickets-like bone changes and increases the risk of mortality (Michaelsen et al., 2009). In adults, phosphorus deficiency may cause osteomalacia, a softening of the bones (Soetan et al., 2010) whereas pregnancy and lactation do not affect the recommended intakes (DRI, 1997).

CONCLUSION

Data presented in this study are of prime importance providing nutrient composition of selected fresh and processed fish species commonly consumed by women and preschool children in Cambodia, both in terms of the number of species and the nutrient components analyzed to date with a specific focus on calcium, iron, phosphorus, protein, fat, carbohydrate, energy, ash and moisture. Our data show that from a nutritional perspective, fish species mostly small species hold the potential to provide a greatly contribution to micronutrient intakes of women and preschool children compared to common aquaculture species. This is likely partially due to the way in which small fish are consumed, that is whole with head and bones. Further still, given the large range in nutrient components of the different species reported here, diversity in fish consumption, particularly of small species, is promising to promote a more all-inclusive nutrient intake. This supports the compelling argument that to effectively target malnutrition, resources should be directed towards ensuring a more balanced approach of both sustainable capture fisheries management and aquaculture, including the development of innovative aquaculture technologies which incorporate nutrient-dense species, in particular small species. This study significantly expands the current knowledge on nutritional value of the great diversity of fish species in Cambodia, and demonstrates that many species, predominantly small species of rice-field fisheries and those from inland capture fisheries, have the potential to contribute significantly to recommended nutrient intakes for a variety of nutrients. In future studies, it would be useful to determine the actual contribution of different species to nutrient intakes of women and preschool children based on consumption, to better inform programs targeting improved access, availability and consumption of nutritious diets.

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TABLES AND FIGURES

Table 1. Identification details of fish samples and anatomical parts removed prior to analysis.

Local/common name	Scientific name	Length (Cm)	Anatomical parts excluded prior to analysis
Fish species			
Trey Chrakeing	*Puntioplites proctozystron*	15.0	Bones, head, scales, viscera, fins, tail
Trey Ta orn	*Ompok hypophthalmus*	17.0	Bones, head, viscera, fins, tail
Trey Changwa phlieng	*Esomus longimanus*	0.7	Viscera
Trey Chhviet	*Pangasius macronema*	14.5	Bones, head, viscera, fins, tail
Trey Kamphlienh sre	*Trichopodus trichopterus*	9.5	Head, scales, viscera, fins, tail
Trey Srakar kdam	*Cyclocheilichthys apogon*	7.5	Scales, viscera, fins, tail
Trey Slek reussey	*Parachela siamensis*	10.0	Head, scales, viscera, fins
Trey Kahe krorhorm	*Barbus schwanenfeldii*	16.0	Bones, head, viscera, fins, tail
Processed fish			
Smoked Trey Changwamoul	*Rasbora tornieri*	-	No parts removed
Smoked Trey Slek reussey	*Parachela siamensis*	-	No parts removed

-, no data available.

Table 2. Summary of nutrient composition of selected fresh and processed fish species commonly-consumed by women and preschool children in Cambodia.

Nutrients	Fresh		Processed	
Proximate components	Min (%)	Max (%)	Min (%)	Max (%)
Protein	14.53	18.82	38.02	50.06
Fat	1.92	13.99	20.18	47.15
Carbohydrate	1.57	8.45	3.29	11.07
Ash	0.78	3.47	6.23	8.94
Moisture	66.83	76.38	5.31	9.76
	Min (Kcal/100g)	Max (Kcal/100g)	Min (Kcal/100g)	Max (Kcal/100g)
Energy	90.18	197.70	426.12	589.65
Micronutrients	Min (mg/100g)	Max (mg/100g)	Min (mg/100g)	Max (mg/100g)
Calcium	126.84	534.48	471.50	2488.25
Phosphorous	116.33	512.50	893.05	1280.62
Iron	1.11	5.11	6.09	6.96

Data are the means of 3 independent experiments, each performed in triplicate.

Table 3a. Details of nutrient composition of selected fresh and processed fish species commonly-consumed by women and preschool children in Cambodia.

Local/common name	Scientific name	Protein (%)	Std	Fat (%)	Std	Ash (%)	Std	Moisture (%)	Std	Calcium (mg/100g)	Std
Fish species											
Trey Chrakeing	*Puntioplites proctozystron*	16.49	1.18	2.02	0.74	1.05	0.38	73.84	3.95	189.30	18.62
Trey Ta orn	*Ompok hypophthalmus*	16.63	2.68	3.14	0.51	1.27	0.30	74.69	2.64	181.75	13.18
Trey Changwa phlieng	*Esomus longimanus*	16.67	3.0	2.39	1.58	3.47	0.33	75.90	0.50	355.69	84.59
Trey Chhviet	*Pangasius macronema*	14.53	2.98	13.99	4.87	0.78	0.08	67.28	5.72	126.84	22.33
Trey Kamphlienh sre	*Trichopodus trichopterus*	18.82	2.61	2.27	0.81	2.40	0.34	74.59	0.72	194.50	27.24
Trey Srakar kdam	*Cyclocheilichthys apogon*	14.93	0.75	1.92	0.19	3.47	0.07	76.38	0.52	534.48	6.22
Trey Slek reussey	*Parachela siamensis*	16.32	0.52	9.79	0.49	2.29	0.10	69.21	0.71	423.65	8.76
Trey Kahe krorhorm	*Barbus schwanenfeldii*	18.0	0.36	5.62	0.23	1.11	0.13	66.83	1.16	512.44	28.0
Processed fish											
Smoked Trey Changwamoul	*Rasbora tornieri*	50.06	3.04	20.18	0.63	8.94	0.30	9.76	1.41	471.50	24.20
Smoked Trey Slek reussey	*Parachela siamensis*	38.02	2.13	47.15	1.51	6.23	0.47	5.31	0.94	2488.25	352.94

Data are the means of 3 independent experiments, each performed in triplicate. Std – standard deviation

Table 3b. Details of nutrient composition of selected fresh and processed fish species commonly-consumed by women and preschool children in Cambodia.

Local/common name	Scientific name	Phosphorus (mg/100g)	Std	Iron (Fe) (mg/100g)	Std	Carbohydrate (%)	Std	Energy (Kcal/100g)	Std
Fish species									
Trey Chrakeing	*Puntioplites proctozystron*	134.0	53.31	2.53	0.23	6.61	3.26	110.53	20.07
Trey Ta orn	*Ompok hypophthalmus*	116.33	23.15	4.42	0.25	4.27	2.91	111.85	11.34
Trey Changwa phlieng	*Esomus longimanus*	474.63	103.52	2.04	0.52	1.57	1.80	94.51	9.46
Trey Chhviet	*Pangasius macronema*	178.76	79.81	2.53	0.69	3.42	2.64	197.70	47.0
Trey Kamphlienh sre	*Trichopodus trichopterus*	353.23	42.71	1.11	0.27	1.93	2.28	103.42	5.81
Trey Srakar kdam	*Cyclocheilichthys apogon*	512.50	22.08	3.05	0.29	3.31	0.73	90.18	2.87
Trey Slek reussey	*Parachela siamensis*	271.68	22.35	3.29	0.43	2.40	0.44	162.93	5.40
Trey Kahe krorhorm	*Barbus schwanenfeldii*	148.32	7.71	5.11	0.14	8.45	1.0	156.34	5.30
Processed fish									
Smoked Trey Changwamoul	*Rasbora tornieri*	1280.62	121.57	6.96	0.33	11.07	2.49	426.12	7.81
Smoked Trey Slek reussey	*Parachela siamensis*	893.05	80.46	6.09	0.39	3.29	2.28	589.65	12.93

Data are the means of 3 independent experiments, each performed in triplicate. Std – standard deviation

IMPLEMENTING AND ASSESSING CELL-BASED TECHNICAL AND MARKETING SUPPORT SYSTEMS FOR SMALL AND MEDIUM-SCALE FISH FARMERS IN UGANDA

Food Safety, Post-Harvest, and Value-Added Product Management/Study/16FSV02AU

Moureen Matuha[1], Isaac Omiat[2], Joseph J. Molnar[3], Gertrude Atukunda[1], John Walakir[a]1, Erina [Nabafu1,] Theodora Hyuha[4], and Claude Boyd[3]

[1]National Fisheries Resource Research Institute (NaFIRRI), Uganda
[2]AgroMarket Day, Ugamda
[3]Auburn University, USA
[4]Makerere University, Uganda

ABSTRACT

The rapid development of mobile technology, the increased penetration rates and the inherent characteristics of mobile phones are the reasons mobile phones are currently emerging as the fast widespread form of electronic communication system in African countries. Mobile phones can, therefore play a key role in disseminating relevant information to fish farmers in Uganda. This report summarizes critical aquaculture needs of the farmers, socio-demographic Characteristics of the farmers, most important anticipated benefits of the app, reviews, comments and suggestions on the design and structure of the aquaculture application. Majority of the participants appreciated the design of the prototype as well as its applications while a few of the participants requested that the look (design) of the app be improved. Information on mixing feeds and feeding, up-to-date fish market, pond management (water exchange, site selection, pond construction, infrastructure development, management, and stocking, input suppliers and buyers' location, predator and health issue, success stories of farmers, booking keeping and fish farming videos were mostly needed by farmers. Respondents' characteristics such as age, gender, and education level were found to be determinants of mobile phone type ownership. Thus, the developed Aqua-application can be used to achieve various activities involved in fish farming and be adopted by policy organizations and government agencies in their proposals on the usage of technology as a key driver towards aquaculture economic growth. However, to leverage the full potential of information dissemination enabled by the designed mobile App will require capacity building amongst farmers on how to effectively use the app, use of different local languages, scaling the app through fish farmers' groups and awareness through different social media. Further work is needed to evaluate the usage and impacts of the app on aquaculture development and the livelihoods of farmers.

INTRODUCTION

Fish farmers in rural and remote areas of Uganda are facing many unprecedented challenges brought on by the changing global economy, dynamic political contexts, environmental degradation, and inadequate information access. To deal with these challenges, and to make critical decisions, fish farmers must be able to access critical information. Access to appropriate farming and market price information, inputs, and technical support are significant determinants for maintaining a successful farming business (World Bank 2013). Farmers need to have access to agricultural information in order to improve their agricultural production (Adomi et al. 2003).

Utilization of available information by farmers is very important (World Bank 2013). Information and communication technologies like mobile phones seem to influence the commercialization of farm products, as a result of easy accessibility of both market and agricultural information by farmers. They have provided new approaches to farmers to make tentative decisions much more easily than before (Ilahiane 2007). The availability of mobile phones can lead to greater social cohesion and improved social relationships.

Mobile phones have also been used in different African countries for a variety of purposes. For example: Aker and Mbiti (2010) identified five potential mechanisms through which mobile phones can be used for economic benefits to consumers and producers in Sub-Saharan Africa. These mechanisms include the use of mobile phones to improve access to and use of information, thereby reducing search costs while improving coordination among agents and increasing market efficiency; an improvement of firms' productive efficiency due to the increase communication by allowing the firms to better manage their supply chains; the creation of new jobs to address demand for mobile-related services, thereby providing income-generating opportunities in rural and urban areas; facilitating communication among social networks in response to shocks, thereby reducing households' exposure to risk; and finally the usage of mobile phone-based applications and development projects to facilitate the delivery of financial, agricultural, health and educational services.

Muto and Yamano (2009) also found out that mobile phone coverage was associated with a ten percent increase in farmer's profitability in the bananas market. In agreement with Aker and Mbiti (2010), it is clear that the introduction of mobile phones in agriculture and other trade sectors has increased the traders' welfare, by increasing their sales prices, as they were able to take advantage of the spatial arbitrage opportunities.

In addition, mobile phones have been used for geo-referenced environmental monitoring. Vivoni and Camilli (2003) developed a wireless prototype system to acquire, store, display and transmit actual geo-referenced environmental data between numerous field teams and remote locations. Motorola Laboratories also developed a system that is used to sense agricultural, environmental and process parameters (Perkins et al. 2002). Lee et al. (2002) also came up with a silage yield mapping system that included a moisture sensor, a Global Positioning System (GPS) and a Bluetooth wireless communication module. Besides spatial data collection, Cugati et al. (2003) developed an automated fertilizer application for tree crops. The system had had a decision module for estimating the optimal amount and spread of fertilizer and an output module to regulate the rate of fertilizer application. The various modules communicated via Bluetooth network.

Furthermore, short Message Service (SMS) system is another form of mobile application that is being widely used in agriculture. Farmers can interact with technical experts via SMS. In Rwanda, the eSoko project was launched in 2009 to allow farmers to access prices of different agricultural commodities via SMS (Hellström2012). An SMS service system that brings information on demand and supply to farmers and extension workers has been developed in Uganda (Hellstrom 2012.). In Ghana, farmers in Tamale were able to send a text message to learn corn and tomato prices in Accra, which is more than 1,000 kilometers away (Aker & Mbiti 2010).

Even though the use of mobile phones have continued to be the best hope for farmers in most of African countries, fish farmers in Uganda still face major challenges including inefficiencies in information delivery, reconciliation of records between farmers and traders, and lack of information on best practices to farmers and timely market price information. Previous AquaFish research identified some of the challenges fish farmers face (Matuha et al. 2016). To deal with these challenges and for farmers to be able to make critical decisions, an aquaculture mobile application has to be developed. This application will help farmers to have access to critical farming and market price information. Therefore, the purpose of this study was to develop, review, launch and introduce the Application to fish farmers, dealers in aquaculture inputs, extension workers, researchers and policy makers and to assess the efficacy of the tool among different users in Uganda.

OBJECTIVES

1. Develop and implement a cell-based system that will enable fish farmers to access fish production and market information.
2. Conduct trials of cell-based aquaculture applications for fish farmers.
3. Conduct trial usage and assessment of cell-based aquaculture applications among the target population of fish farmers.
4. Evaluate utilization of cell-based applications for technical support, marketing and input discovery among the target populations of fish farmers.
5. Introduce mobile-based application to the network of agencies and organizations that support aquaculture.

MATERIALS AND METHODS

Activity one: focused group interviews

This effort developed and implemented a cell-based system enabling fish farmers to access fish production and market information. A total of 45 fish farmers, comprising of 34 men and 11 women, participated in the focus group interviews. The study population included small and medium-scale fish farmers from five districts (Wakiso, Mpigi, Gulu, Kole and Kalungu) of Uganda (Table 1). The interviews were conducted during the months of July to September, 2016.

The guided conversations were conducted mainly to assess the socio-demographic characteristics of farmers, identify critical aquaculture information needs and anticipated benefits of using the designed mobile application platform. Secure and quite environments were created, which allowed participants to feel comfortable to share their views, beliefs and attitudes without the fear of judgment from others. All the discussions were conducted in the indigenous languages spoken by people in the study areas and each lasted one and half hours. The discussions were digitally tape recorded in addition to manual note-taking of key issues. The data were transcribed, verified and coded using ATLAS. ti (Version 8). The codes were then exported into a computer Statistical Package Statistical Package for the Social Sciences (SPSS) (version 21), which yielded descriptive statistics such as percentages, means, and frequencies.

Activity two: trials of cell-based aquaculture applications for fish farmers

In order to improve the design and application of Aqua-App on both android smart phones and the basic phones, an IT Technocrat from Likamis Software demonstrated the design and structure of the app (Figures 1&2) to researchers, farmers, traders, and representatives from NARO, MAAIF and other governmental agencies. Demonstrations were carried out in March and May 2017 during two workshops organized at Kajjansi Aquaculture Research and Development Centre (KARDC). The workshops aimed at training the participants on how to use, review and improve the design of the App according to issues raised by participants. A total of 35 participants attended the workshops. Immediately after the demonstration, participants were allowed to evaluate the design and applications of the App; ask questions; make suggestions; comments and inputs. Information from the evaluation and suggestions records were entered into Microsoft Excel 2013 and analysed.

Activity three: introduce mobile-based application

To introduce the applaction to the network of agencies and organizations that support aquaculture several different efforts were made. Following the reorganization of the modules of the app, a presentation was made by one of the IT staff of Likamis Sofware to farmers, buyers, processors, and government and non-government agency representatives during the Fish Farmers' Symposium that was held in January 2018. The workshop aimed at introducing and equipping users with skills on how to download, subscribe and use the application on their mobile phones. The mechanisms for continued sharing of aquaculture information among farmers, researchers, policy makers and ICT professionals were emphasized. This meeting was also a central aspect of the project exit strategy in Uganda.

RESULTS

Activity one: focused group interviews

Characteristics of respondents:

Out of the 45 respondents, 33(75.56%) were males, while 11 (24.44%) were females (Table 2). Close to half of the respondents (48.89%) owned smart phones while the rest had basic phones. Smart phones give farmers' access to millions of Apps and saves a lot of their time than basic phones. Furthermore, of the 45 respondents, 40 (88.89%) were young, aged between 25-45 years old. This implied that over two thirds of the young respondents owned mobile phones. Such findings agree with Souters et al. 2005 who found that majority of Information Communication Technology users tends to be young adults. In terms of education, most respondents, 15 (33.33%) were secondary school leavers, 27 (60%) had either certificate or diploma education and 3 (6.67%) had attained university education. These findings show that all these categories of farmers are in position to use the app.

Critical aquaculture information packages/topics:

In terms of the kind of information that should be included on the app, most farmers indicated that the development of the app is a good initiative and that it will go a long way in easing access to information needed to advance fish production. Table 3 shows the information topics that were emphasized by farmers for inclusion on the app during the designing process. The interviews showed that information on mixing feeds and feeding (32%) was the mostly needed by farmers, followed by providing information on up-to-date fish market prices (25). 19.5% of the participants indicated that pond management (water exchange, site selection, pond construction, infrastructure development, management, and stocking) is very vital for a farmer and that this should not miss on the app, 14.2% of the participants indicated that all farmers, input suppliers and buyers' location and contact information should be included on the app, 12% of farmers emphasized information on predator and health issue management. 10.3% preferred information on success stories of farmers and booking keeping while 9% indicated that fish farming videos should be included on the app for farmers to download and watch at their own convenience

Anticipated benefits of using the designed mobile applications in aquaculture:

Farmers anticipated that the use of use mobile phone application to be designed could confer diverse benefits as a communication link in isolated areas because of its distinct feature of mobility. Table 4, shows that about third (1/3), (33.3%) of the respondents reported that, the mobile application to be designed would greatly help farmers to easily obtain fish production and market information. Respondents also named a number of virtues that would be associated with the use of a mobile phone app. For example, during the discussions, it was pointed out that the use of mobile app would help farmers to discuss prices with buyers and crosscheck prices for their produce, instead of relying on middle men/brokers (24.4%). This will assist them to make better choices on where and when to buy or sell their farm produce. The interviews further indicated that farmers would be in best position to exchange information at their convenience (20.2%).

Mobile phones were therefore, reported as a good delivery tool that would save fish farmers' time and costs associated with travelling long distances to procure farm inputs and other related activities (13.3%). Likewise, Jensen (2007) asserted that mobile phones have an ability to save farmers' costs, by providing quick access to agricultural information, communication with trade partners and opens new market possibilities. On the other hand, farmers mentioned that the mobile app would help them make timely decisions (8.8%).

Activity two: trials of cell-based aquaculture applications for fish farmers.

Aqua-application evaluation

Majority of the participants appreciated the design of the App as well as its applications especially that of the USSD phones. A few found the App very easy, especially when checking, contacts, price and checking sellers' details (Figure 3).

Suggestions from participants

Sixty five percent of the participants were satisfied with the design and application of the prototype. The participants noted the App will be useful to the farmers and further requested that the appearance of the app should be improved (Figure 3). Eleven percent suggested improving functionality of the application whereas the remaining 10% suggested that the fish production information be improved.

Activity three: introduce mobile-based application

For example, members suggested that Modules (site selection, pond construction, infrastructure development, management, and stocking) should be merged into one broad topic named management. The merger would create more space for inclusion of information on other production systems like cages and tanks. In addition, participants suggested the app should only focus on tilapia and Catfish, the species mostly cultured by Ugandan farmers. Basing on suggestions by participants, modules were re-assessed, improved and reorganised. The overall functionality of the App was improved with features such as ability to add commodities, buyers and input suppliers, how to compare prices, ranking of prices and classifying by area.

CONCLUSION

The study established that mobile phone Aqua-Application acceptance to fish farmers is high and e to accompany it with a predictable positive economic impact. Therefore, most fish farmers anticipated that the designed mobile application is a good technology that will mostly enable them to have proper access to timely fish production and market information since these are the major bottle-necks to Ugandan aquaculture. Information on mixing feeds and feeding, up-to-date fish market, pond management (water exchange, site selection, pond construction, infrastructure development, management, and stocking, input suppliers and buyers' location, predator and health issue, success stories of farmers, booking keeping and fish farming videos were mostly needed by farmers. Respondents' characteristics such as age, gender, and education level were found to be determinants of mobile phone type ownership in the study area.

The Aqua-App is being used to carry out various business activities involved in aquaculture. The app currently allows more contacts amongst farmers, enables exchange of information any time the need arises. In addition, the app enables making contact with customers easily, saves time and other transaction costs as well as eliminating brokers. The farmers are also able to get daily market prices of agricultural commodities across the entire country. Producers are able to price their products appropriately, and can learn better practices conducted in aquaculture production

The design could also be adopted by policy organizations and government agencies in their proposals on the usage of technology as a key driver towards aquaculture economic growth. It is evident that the developed prototype presents a workable solution towards the design and implementation of mobile applications in fish farming as this will help to improve on the aquaculture development of Uganda. Researchers can use the designed prototype as a basis for improvement towards existing mobile application development models and frameworks or to develop new ones. However, there is need to popularize the app among different categories of people (extension workers, input dealers, traders, processors, and farmers), conduct workshops in different regions of Uganda about the use of the app, continuous monitoring and evaluation on order to track the level of use and evaluate the impacts of the use of Aqua-App on the livelihoods of farmers and aquaculture development in Uganda.

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TABLES AND FIGURES

Table 1: Distribution of focused group interviews with fish farmers, Uganda

District	Meeting location	Number of participants	Gender	
			Males	Females
Wakiso	District Council Offices	13	8	5
Mpigi	District Council Offices	10	7	3
Kalungu	Former District Council Offices	7	7	0
Gulu	Sub-county offices	8	7	1
Kole	Sub-county office	7	5	2
Total		45	34	11

Table 2: Socio-demographic characteristics of respondents (N=45)

Characteristics	n	%
Male	34	75.56
Female	11	24.44
Respondent's age		
Young (($\leq$ 45 years)	40	88.89
Old ((> 45 years)	5	12.11
Educational level		
Primary/Secondary education	15	33.33
Certificate education	9	20.00
Diploma education	18	40.00
University degree	3	6.67
Type of phone owned		
Smart phones	22	48.89
Basic phones	17	37.78
Both smart and basic phone	6	13.33

Table 3: Aquaculture information topics suggested for inclusion on the app (N=45)

Variable	Percentage (%)
Mixing feeds and feeding information	32
Up-to-date fish market prices	25
Pond management (water exchange, site selection, pond construction, infrastructure development, management, and stocking	19.5
Fish farmers, input suppliers and buyers' location and contact information	14.2
Predator and health issue management information	12
success stories of farmers and booking keeping guidelines	10.3
Fish farming practice videos	9

Table 4: Most important anticipated benefits of using the designed mobile applications (N=45)

Variable	n	%
Help farmers to discuss prices with buyers	15	33.3
Help farmers to easily get fish farming and market information	11	24.4
Exchange information at their convenience	9	20.2
Allow more contacts amongst farmers	6	13.3
Saves time and costs in dealing with related activities	4	8.8
Total	45	100

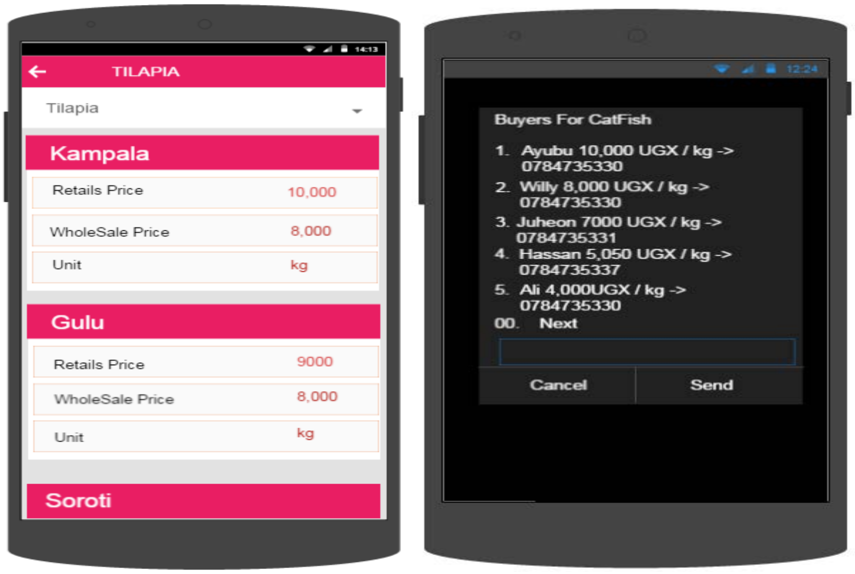

Figure 1: Market price information for different buyers in different locations

Smartphone USSD phone

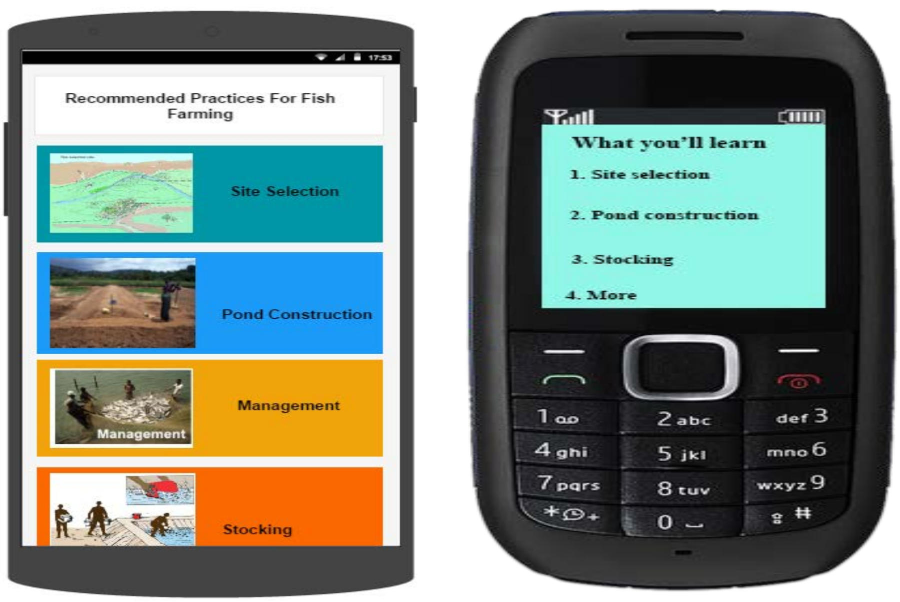

Figure 2: Fish farming information

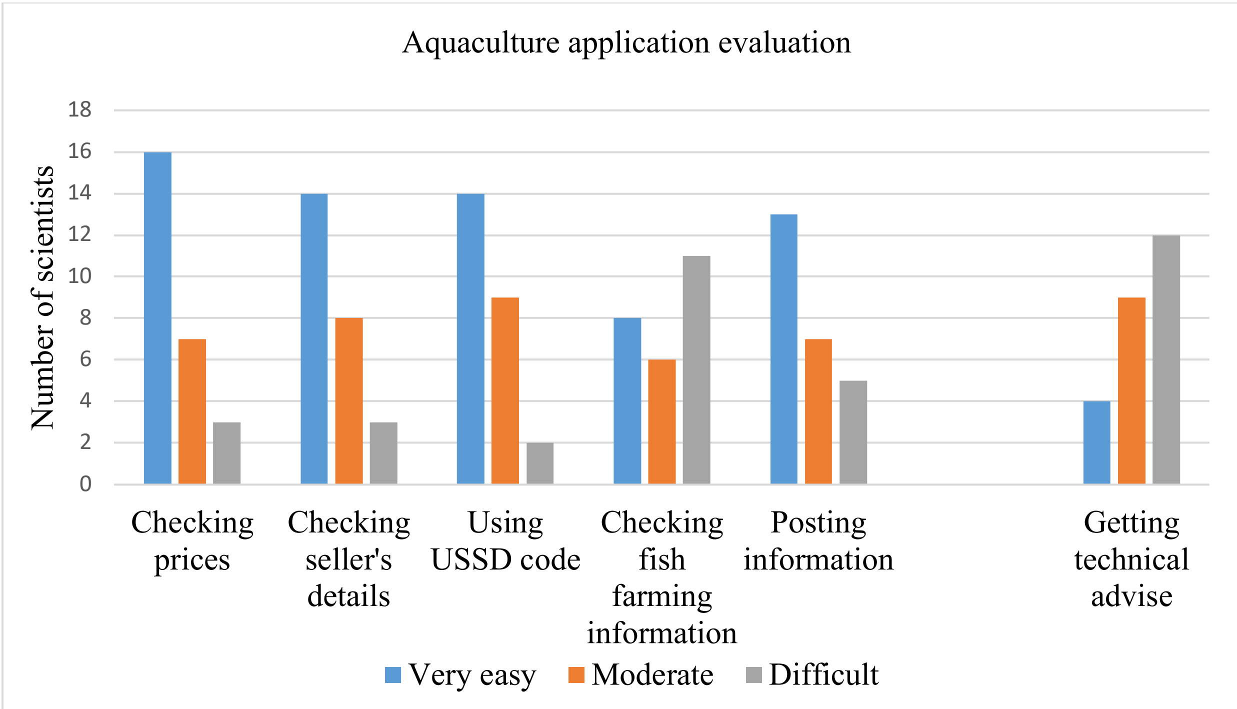

Figure 3: Aqua- application evaluation

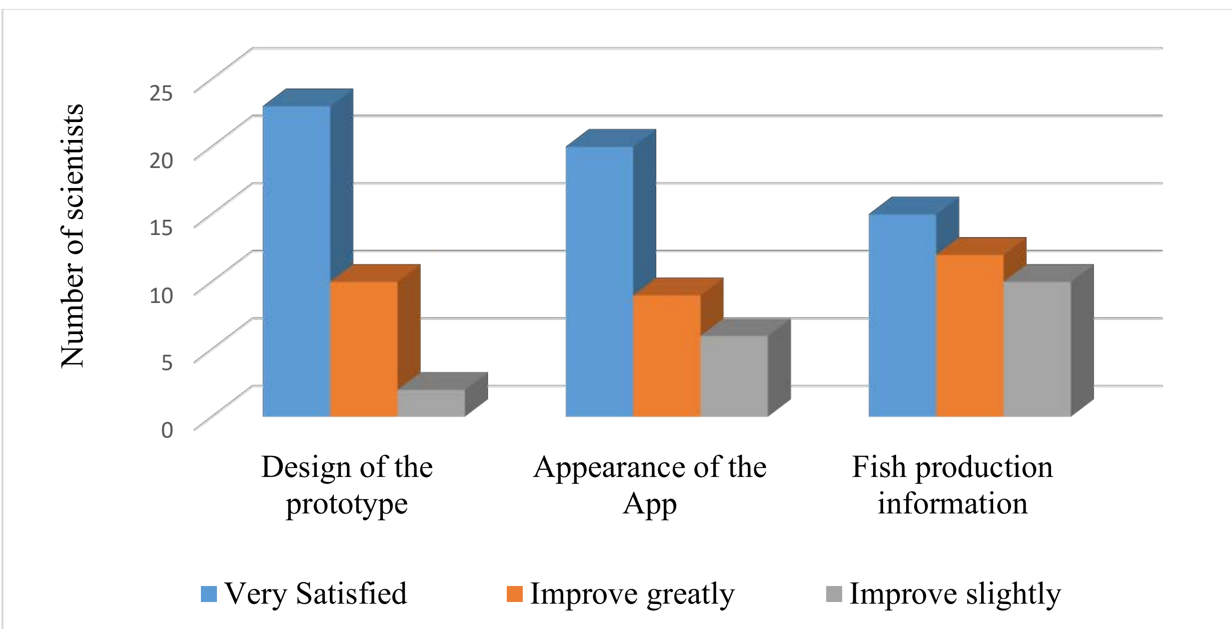

Figure 4: Suggestions from participants

TOPIC AREA

POLICY DEVELOPMENT

❖

GUIDANCE AND POLICY RECOMMENDATIONS FOR SUSTAINABLE SNAKEHEAD AQUACULTURE AND AQUATIC RESOURCE MANAGEMENT IN CAMBODIA AND VIETNAM

Policy Development/Activity/16PDV01UC

Hap Navy[1], Huynh Van Hien[2], Nguyen Thi Kim Quyen[2], Dang Thi Phuong[2], Tran Dac Dinh[2], Nguyen Thanh Long[2], Truong Hoang Minh[2], Tran Thi Thanh Hien[2], Pham Minh Duc[2] and Robert Pomeroy[3]

[1]*Inland Fisheries Research and Development Institute, Cambodia*
[2]*College of Aquaculture and Fisheries, Can Tho University, Vietnam*
[3]*Department of Agricultural and Resource Economics/CT Sea Grant, University of Connecticut-Avery Point, Groton, CT, USA*

ABSTRACT

Investigation 2 was conducted with the goal to clarify and provide science-based guidance and policy recommendations related to stakeholders involved in snakehead industry for sustainable snakehead aquaculture in Cambodia and Vietnam. The research includes four activities: i) A desk-study on cost-profit in different snakehead farming models and scale based on previous study; ii) Audience analysis; iii) Reviewing project products and consulting experts to extract key message; and iv) communication and dissemination strategy. The study was based on previous research undertaken under the AquaFish CRSP and AquaFish Innovation Lab in Cambodia and Vietnam that focused on value chain analyses to provide more in-depth understanding of cost and profitability of snakehead culture. The results of activity 1 shows that cage and pond culture system were both commonly used in Cambodia. The results of the study in 2006 indicated that snakehead cage culture had higher profitability compared to pond culture system, USD 570 per cage per cycle and USD 29 per pond per cycle, respectively. There was similar results from the study in 2011, the net profit for small scale cage culture was negative, while medium scale had a gain in profit of USD 4,353. For pond culture, the net profit for small, medium, and large scale were all negative. Both cage and pond culture systems in 2016 show loss in net profit. Cage culture farmers lost approximately USD 336 per cage per cycle and for farmers who applied pond system, they lost about USD 11,491. The major contribution that led to increases in operational production cost for both cage and pond culture system in Cambodia and Vietnam derives from feed cost. As clearly seen in study in Cambodia, the highest costs for snakehead cage and pond culture operation came from fish feed which contributes about 81% for cage and 75% for pond, respectively. The cost benefit analysis of fish cage culture indicates that the gross return per cage unit was 24,800,000 Riel (USD[3] 6,200) per cycle of fish raising, whereas the net profits was 3,310,000 Riel (USD 828). This figure excludes salary or wage of labor. However, on including the cost of wage labor, the net profit stands out at 1,510,000 Riel (USD 378),

[3] Assuming the exchange rate is : 1 USD = 4,100 Riel

which is about 54% of the total net profits. Of the total input costs, the highest costs for fish culture operation was fish feed, which contributes to about 74% of the total costs. This implies that the fish cage culture operation was profitable and is expected to continue in business. This suggests that the fishing household should have at least one cage culture in each household for improving their income and living condition.

In Vietnam pond culture and hapa were popular systems. Currently, almost all pond farms and nearly 90% of hapa model used pellet. Regarding scale production, pond culture with medium scale (1,000 – 2,000 m^2) brought highest profit (30 – 40 ton/farms and 11.31 USD/m^3/crop). In hapa system, farmers could be most profitable with 1.42 – 9.76 USD//m^3/crop (hapa in pond) and 1.29 – 10.9 USD/m^3/crop (hapa in river). Training-workshop organized in activity 2 revealed that diversity in snakehead culture models was still necessary for different farmers. Seed quality, disease management, and market function were key challenges. Support from authority in anticipation of market equilibrium production was requirement. Activity 3 also gave pragmatic recommendations, including Policy brief, guidelines, poster, leaflet and factsheet. Such materials were disseminated to propaganda and appropriate beneficiary in activity 4.

INTRODUCTION

Snakehead is a typical species of fresh water, which is raised in the Mekong Delta (MD) and cage culture in Cambodia. This species is considered as an easy species to grow with different models and scales (Sinh et al., 2009). Snakehead fish farming systems in the MD are now mainly earthen pond and hapa (in pond and in the river). Additionally, fish are also raised plastic tank and cage. The two main snakehead species currently cultured are *Channa striata* and *Channa micropeltes* (accounting for 20%) (Chung and Sinh, 2011). Snakehead is also chosen for small-scale production for the purpose of reducing poverty and replacing the ever-decreasing natural snakehead (Sinh et al., 2009). Snakehead culture are concentrated mainly in An Giang, Dong Thap, Can Tho, Hau Giang, Vinh Long and Tra Vinh provinces. Production increased rapidly from 5,300 tons in 2002 to 40,000 tons in 2010 (Long et al., 2004; Chung and Sinh, 2011). Snakehead is also chosen for small-scale production for the purpose of reducing poverty and replacing the ever-decreasing natural snakehead (Sinh et al., 2009). Within the freshwater aquaculture model in Cambodia, cage culture represents the highest percentage of about 70% of aquaculture production, while pond culture covers only 30%. The most important and highest profit fish species in cage culture system in Cambodia is Chhdaur (Giant Snakehead, Channa Micropeltes) (So et al., 2005). Giant snakehead is commonly raised in cages in and along the Mekong River, the Great Lake Tonle Sap and Tonle Sap River (Hap and Pomeroy 2010).

In April 2016, the Government of Cambodia lifted the decade-old ban on snakehead fish farming following a request from the Ministry of Agriculture to allow farmers to fish. Signed by Bun Uy, a secretary of state at the Council of Ministers, the statement said the decision to legalize snakehead fish farming again would be accompanied by forthcoming conditions and advice for farmers. In the statement, Mr. Uy said the conditions and advice, to be issued by the Agriculture Ministry, would help farmers sustainably manage and maintain their farms, and keep fish stocks healthy.

Previously, snakehead was usually fed with fresh feed, mainly marine small-size fish, freshwater small-size fish and yellow snail (Chung and Sinh, 2011). The use of small-size fish/low value fish as directly feed to snakehead fish farming has caused environmental problems, increased pathogens leading to mass death due to poor water quality (So Nam and Robert Pomeroy, 2011). In addition, the conflict in the use of small-size fish for aquaculture and food consumption is increasingly as less and less fishing yields of small-size fish that lead to increase in price of small-size fish (Navy and Pomeroy, 2009). These circumstances have driven many studies in pellets aimed to replace small-size fish in snakehead fish farming. The success of complete conversion to pellet feed has contributed to the rapid development of aquaculture, productivity increase and poverty reduction (Sinh et al., 2009). However, the significant increase in snakehead production has led to sharp fluctuation in market price from 2013. In addition, climate change, rising temperature and salinity intrusion also affected considerably to snakehead fish farming, especially the increase in investment cost while market price was still low that leaded to losses in recent years (Tran Hoang Tuan et al., 2014). Therefore, the review of cost-benefit efficiency along with changes in farming techniques of different models and scales of snakehead farming is necessary to see the trend of development, find out the success stories and issues as well as proposing necessary policies for sustainable snakehead farming in the Mekong River.

Therefore, this investigation utilizes this broad research on snakehead aquaculture to support the development of sustainable snakehead aquaculture in Cambodia through research based on guidance to farmers on feeding, breeding, weaning and rearing/grow-out. The lifting of the snakehead ban in Cambodia in April 2016 will allow for enhanced trade and investment for Cambodian farmers as snakehead is in high demand both domestically and regionally and there will be investment in feed mills, grow-out operations, processing and other post-harvest activities.

OBJECTIVE

The objective of this activity is to provide science-based guidance and policy recommendations to government and farmers and households, including vulnerable subpopulations such as women and children, on sustainable snakehead aquaculture in Cambodia and Vietnam.

MATERIALS AND METHODS

To support sustainable snakehead aquaculture, there is a need to provide science-based information to government, households and vulnerable populations in order to be able to make informed and deliberate decisions on snakehead aquaculture. As an activity, the purpose is not to generate new information but to disseminate and communicate information generated by the studies in the project. Specifically, science-based guidance and policy recommendations. This investigation will provide the information through a suite of different communication methods and approaches for each audience.

Key activities will include:

Activity 1. To examine the cost and profitability for snakehead culture in different production systems in Cambodia and Vietnam. These activities were carried out on previous research undertaken under the AquaFish CRSP and Innovation Lab in Cambodia and Vietnam that focused on snakehead value chain analyses to provide a more in-depth understanding of cost and profitability of snakehead culture. The activities were focus on snakehead culture in cage and pond production systems at different scales of production (small, medium, large). An extensive review of past studies was carried out in relation to production cost and profitability of snakehead culture in Cambodia and Vietnam. The review and desk-based research were help to establish the nature of costs involved in each type of production system i.e., categories of capital and labor used in snakehead culture. Specific information to be obtained will include investment cost, operational/variable input costs (seed, feed, chamical and drug), fixed costs (insurance, permits, license, tax, interest rate on borrowed fund and repair/maintenance etc.), total cost, selling price, gross and net income.

Synthesis method in activity 1 as follow:

- ***Step 1*:** To gather all related AquaFish and general papers/articles, including 27 papers in Vietnamese version and 7 previous reports from the project.
- ***Step 2*:** To set up review framework, research team proceeded separation and reviewing papers. Summary cost and profit in snakehead culture during different periods was conducted and filled of framework after that.
- ***Step 3***: Preparing report based on reviewed information. This report will be presented for activity 2: Audience analysis.

Activity 2: Audience analysis. The identification of target audiences including scientists, researchers, resource managers, government officials, NGOs, farmers, women and their specific information requirements and methods of receiving information (workshops, trainings) and appropriate communication products (e.g. policy briefs, technical report, journal articles, web media) and the style of communication including scope, where and how to receive information, language, technical content. Focus Group Discussions (FGDs) will be conducted with each audience group to identify appropriate communication channels for information dissemination and their preferences of communication channels.

In Cambodia, three FGDs were conducted in Battambang (21 June 2017), Kompong Thom (22 June 2017) and National level at the Inland fisheries research and development institute, Phnom Penh (11 July 2017). In Vietnam also three FGDs were conducted in An Giang (19 April 2017), Tra Vinh (26 April 2017) and Can Tho (9 May 2017) under the name of "Workshop-training on snakehead culture development sustainability based on audience analysis". The total number of participants for Cambodia was 138 which was divided into three groups (Table 1): Group delegates from farmers (Small, Medium and large); Group delegates from private sector (Hatchery/Nursing, Feed and chemical company processor and trader) and Group delegates from government official (aquaculture and fisheries official, researcher and policy maker and Chan Tho university, Vietnam). In Vietnam there were 140 participants (Table 5). The Objective of the workshop was to propose solutions for sustainable snakehead culture. All Delegates were divided into three groups: Group 1: Delegates from feed processing companies; Group 2: Delegates from management offices, researchers, experts and NGOs; Group 3: Fish culture farmers, snakehead seed producers, traders and snakehead processing facilities. The workshop followed three steps:

- ***Step 1***: participants were introduced the information on cost-profit in snakehead culture which taking out from activity 1.
- ***Step 2:*** participants were divided into three groups to discuss about three groups of indicators as follow:

Technical indicators:

Interpretations	**What is the present in the locality?**	**What is difficulties? Or shortage?**	**What is a proposed solution? Who will do it? How to apply?**
1. Seed production (broodstocks, nursing, quality)			
2. Farming models (Pond, hapa,tank….) land use?			
3. Scale (small, medium, large)?			
4. Farming technique			
5. Feeds and drugs/chemicals			
6. Environment & diseases?			
7. Others ………			

Market indicators:

Interpretations	**What is the present in the locality?**	**What is difficulties? Or shortage?**	**What is a proposed solution? Who will do it? How to apply?**
1. Markets (domestic and export)?			
2. Raw materials for processing? Processing capacity? Processing products? Processing technique?			

3. Distribution channels?			
4. Market price?			
5. Others ………..			

Political indicators:

Interpretations	**What is the present in the locality?**	**What is difficulties? Or shortage?**	**What is a proposed solution? Who will do it? How to apply?**
1. Planning			
2. Investment from government and private sector?			
3. Extension?			
4. Labors?			
5. Production cooperation? (cooperatives)			
6. Investment attraction?			
7. Others ………..			

Proposing communication products and how to approach products

Necessary communication products	**Approached method**	**Places to approach products**
1.		
2.		

- ***Step 3:*** One person on behalf of each group presented their discuss result and recommendation.

Activity 3. Project products. The project documents from all of the activities of the CRSP and Innovation Lab projects were reviewed, and team members consulted to extract key messages to be presented in the different communication products.

Activity 4. Communication and dissemination strategy. A communication strategy was formulated and implemented by the host country partners. The communication strategy is a combination of approaches, techniques and messages to reach different audiences. Printed media such as policy briefs, guidelines, posters, leaflets and flyers will be developed for dissemination. At a minimum, the strategy will aim to effectively disseminate the results of the following to key audiences.

RESULTS

Activity 1: Cost-Profit Analysis

Cambodia was divided into two different system, include pond and cage in the river or lake. For each system, it was divided into three scale including small-scale, medium scale and large scale. In general, farmers were culturing the snakehead in cage and pond within small, medium and large scale. Cage size is varying from (1x2x1.5) for small, (3x5x1.5) for medium and (5x10x2) for large scale. It is made by bamboo and woods that floating in river, whereas pond size is varying from ≤500m^3 (10x20x2.5) for small, 500 m^3 - 750m^3 (15x20x2.5) for medium and ≥750m^3 for large scale.

Table 2, 3 and 4 shows about the costs and profitability of snakehead culture (excluding salary and wage of labor) in 2006, the average gross return of cage culture was USD 3,588 per cage per cycle, and USD 356 for pond system. Moreover, the study in 2011, indicated that the average gross return for cage culture was USD 4,811 per cage per cycle and USD 3,843 for pond system. Concerning small and medium-scale cage, the average gross return was about USD 1,746 and USD 7,874 respectively, whereas for pond culture, there were USD 2,016 for small, USD 3,922 for medium and USD 5,892 for large scale. With regard to in study in 2016, the result shows that farmers cultured snakehead fish in cage system received gross return about USD 4,233 in average, while in pond system they could get average gross return approximately USD 9,750.

In 2006, the average gross profit of cage culture per cycle was USD 840 and for pond culture was USD 86. Regardless scale, in study in 2011, it reveals that the average gross profit of cage culture was USD 2,250 and for pond system was USD 461. This profitability varies depending on scale of culture operation. For cage system, the medium scale operation had high gross profit (USD 4,951.3 per cage per cycle), whereas the small-scale system reveals loss in terms of gross profit (USD -452 per cage per cycle). This might be due to the high expenditure on feeds and fingerlings which were generally bought at higher price than large-scale farmers. For pond culture, the gross profits for small, medium, and large scale were USD 238, USD 100, and USD 1,046, respectively. Based on the result of the study in 2016, the average gross profit of cage culture was USD 217. However, for pond system, the gross profit was lost (– USD 9,511) because of big expenses on feed cost.

In terms of net profits, showed that cage culture system seems had higher profitable than pond system, that is USD 570 per cage per cycle and USD 29 for pond per cycle. There was similar in 2011, which showed that the net profit for cage culture for the small scale turn to loss (USD -747), while medium scale was USD 4,353. For pond culture, the net profit for small, medium, and large scale was USD -153, USD -71, and USD 383 respectively. This implies that medium scale of cage culture and large scale of pond had more profitability.

In 2016, both cage and pond culture system showed that the net profit was negative. This may due to after lifting snakehead culture in Cambodia, there was many snakehead farmers in Cambodia lead to have surplus of snakehead production lead to have low price.

In Vietnam, snakehead culture was divided into different system; include pond, hapa in pond and hapa in river. For each group, the scale was divided by small-scale, medium scale and large scale. Each model and scale would be characterized by a corresponding system, including hapa in river for small-scale; medium scale for hapas in pond model and pond model was large scale.

The results from Table 6 showed that, small scale (hapas in river model) had an average volume of 56 m^3/hapa and stocking density of 129 ind/m^3. The FCR ratio was 1.42 (pellets feed) and 4.2 (small-size fish feed). The yield reached 32.5 kg/m^3. Production cost was 42.18 USD/m^3/crop, corresponding to 1.3 USD/kg and the selling price ranged 1.34-1.63 USD/kg. Revenue gained 43.48 USD/m^3/crop, corresponding net profit was 1.29-10.9 USD/m^3/crop. Rate of return was 3.1-25.8%.

Cost and profit analysis of medium scale (hapas in pond) from the Table 7 showed that, volume average was 61.5 m^3/hapa with stocking density of 94.2 ind/m^3. The FCR ratio was 1.32 (100% pellets feed). The average yield was 92.2-32.5 kg/m^3. Production cost/crop and per kg were 39.58 USD/m^3 and 1.36 USD, respectively. With the selling price ranging from 1.4-1.66 USD/kg, average revenue could be 41.0-53.8 USD/m^3/crop. Net profit was 1.42-9.76 USD/m^3/crop, equal to 0.05-0.3 USD/kg. Rate of return was 3.6-22.1%.

Turning to pond culture model (large scale), average volume was 3,861 m^3/pond with stocking density of 21.9-50 inds./m^3. The FCR ratio was 1.31 (100% pellets feed). The average yield was 16.2-30.0 kg/m^3. Production cost was 20.81 USD/m^3/crop, corresponding figure per kg was 1.20-1.30 USD/kg. Market price ranged between 1.23 and 1.33 USD/kg. Revenue and profit was 21.4-26.1 USD and 0.57-4.65 USD/m^3/crop (respectively) whereas profit per kg was 0.04-0.29 USD/kg. Rate of return was 2.7-21.0% (Table 8). Financial efficiency by production scales (Figure 1) showed that, medium scale (volume 1,000-2,000 m^3) had the most profitable (USD 11.31/m^3) and production of 30 – 40 ton/hh.

Activity 2: Audience analysis: Sustainable development of snakehead culture in Vietnam
Seed production (broodstocks, nursing, quality): Available seed production systems and supply were very good, the famers were satisfied with quality and quantity of fingerlings (80% respondents). Participants said that nursing techniques resulted high survival rate (50-60%). Broodstocks tended to be bad quality at present time, thus, improving quality, especially genetic terms was proposed by majority of respondents (75%).

***Priority models and suggested scale*:**
In Cambodia, the model of snakehead culture system consists of 2 main system were cage in river, reservoir and lake and pond culture system. The culture system were divided into three categories, including small, medium, and large-scale. For cage culture system was determined that small scale ≤ (1x2x1.5 or 3 m^3), medium scale ≤ (3x5x1.5 or 22.5 m^3), and large scale ≤ (5x10x2 or 100 m^3), while pond size is varying from ≤ 500m^2 (10x20x2.5) for small, 2000m^2 -750m^2 (15x20x2.5) for medium and ≥ 750m^2 for large.

In Vietnam, diversified models of snakehead in the MD were clarified, including hapa in river; hapa in pond, pond, tank, etc. Participants said that each farming model had curtained both advantages and disadvantages. Specifically, the small-scale hapa in river model was relatively suitable for the poor (76% respondents). Each farm should operate 2 hapas which resulted in the most profitable. In hapas in pond model, medium scale should be tackled to improve household economics. Whereas the pond culture model was suitable for large scale investment toward an industry-oriented snakehead culture sector. However, unstable market price has been a significant disadvantage of such scale.

Farming technique: Advanced science and modern technology have been applied for snakehead farming model i.e. VietGAP and circle water culture model. Lack of certification standards and best practice in snakehead culture were a problematic.

Feeds and drugs/chemicals: Cambodia during dry season (October to May) the most important source of feed were freshwater small-sized or low-value fish, while low-value or small sized marine fish species are used during rainy season (June to September). Many types of chemicals were used for snakehead culture. For Vietnam there are also many types of feed and chemicals supply for snakehead culture farming. However, there is no specific feed and chemicals for snakehead as well as lack of food and chemicals quality control method (85% respondents).

Environment & diseases*:* For environment issue, both Cambodia and Vietnam tends to degradation resulted in industrialization and intensive agricultural production, including snakehead culture. Diseases of snakehead appeared more frequently and more difficult to treat (i.e.. white disease). Need more training on disease prevention and treatment for snakehead culture famers from the authority was desire from farmers.

Markets (domestic and export)*:* Cambodia farmers has poor/lack of market network/information, lake of cooperation with each other, lack of information on market price and also the market challenges with the fishes import from Vietnam. For Vietnam the mainly market of snakehead was consumed domestically in Binh Dien wholesale market (70% of output), local markets, supermarkets, and processing agencies (dried and salted products). A small output of snakehead is exported to Cambodia.

Raw materials for processing and processing technique: Both Cambodia and Vietnam, raw fish was provided sufficiently for processing agencies, but the quality was not uniform/standard. Processing technology has still under modernization, products are not diversified and lack of added value products.

Distribution channels: There were about 40% of snakehead production was consumed in the MD and 60% was consumed outside of the region.

Market price: Selling price was unstable. The results from Figure 2 show that selling price on market fluctuated sharply throughout months and years.

Planning: There is no detailed planning on the area of snakehead culture that lead to difficulties in determining quantifies production. It is necessary to make detailed statistics on the area and output of snakehead culture to regulate supply and demand of the market. That is prevent the price dropping so low that fish farmers lose profit (90% respondents).

Investment from government and private sector**:** Lack of synchronous investment by the state in infrastructure for snakehead culture farming. There should be incentives for private investment in snakehead supply chain (60% ideas from participants).

Extension**:** Participants said that the extension system is very good (90% ideas). However, experience of expertise does not meet the demand for technical support to the famers. Therefore, it is necessary to improve the level of expertise for local extension.

Labors**:** Labor in rural areas is abundant and the famers had satisfactory about labors for demand snakehead culture. However, there is a lack of skilled labor in pathology in snakehead culture.

Production cooperation: In Tra Vinh province, there is a cooperative between snakehead farmers and processing company that shows efficiency. It is necessary to build a model linking farming - processing and consumption as well as branding for snakehead.

Activity 3:

1. ***Policy briefs-Main focus on:***
   a) Policy Brief_Impact of Climate Change on Snakehead culture in Cambodia- Translate in Khmer
   b) Developing sustainably small scale snakehead farming to improve nutrition and income for the poor in rural area;
   c) Improving the quality of broodstock and fingerlings for disease and climate change adaptation, orientation to sustainable development;
   d) Encouraging investment in feed processing technology for snakehead;
   e) Improving current policies to develop snakehead following the chain linkages and building the trade mark globally in the future.

2. Guidelines/best practices: best practices documents on sustainable snakehead aquaculture: recommendations for best practices of snakehead pond culture

- The stocking season should be from September to October.
- Good ponds average size is 1,000-2,000 m^2
- Farms should take area for waste treatment.
- Stocking density: 40-80 Inds./m^2.
- Pellets feed: 40-42% protein
- Controlling FCR at 1.2-1.3

- Local workers and the poor should be priority in large-scale farming
- Farming period/crop: 6-7 months

3. Poster

- Genetic diversity of striped in Cambodia.
- To deliverable dissemination of sustainable development of snakehead culture in Vietnam.

4. Leaflet

- Enhancing food security and household nutrition in Cambodia.
- Guidelines and policy suggestions for sustainable snakehead culture and management of aquatic resources in Cambodia and Vietnam

5. Factsheet

Policies to support the sustainable development of snakehead culture in the MD.

Activity 4: Communication and dissemination strategy

The products of the project have been transferred to related parties for propaganda purposes of project on "Guidance and policy recommendations for sustainable snakehead aquaculture and aquatic resource management in Cambodia and Vietnam".

The following audiences: (i) Snakehead feed; (ii) Snakehead feeding strategies; (iii) Processing and value-added products for women; (iv) Improvements in the trade and value chain for both capture and culture fish in the region; (v) Human nutrition and human health impacts of fish; (vi) Wild and hatchery-stocked brooders of snakehead stocking and conditioning; (vii) Snakehead breeding, weaning, and rearing/grow-out; (viii) Economics of production.

DISCUSSION

The results of cost and profit analysis in Vietnam showed that the small-scale model of snakehead culture was suitable for the poor with aim to improve nutrition and used idle labor of household to improve income. Low financial investment and low production risk are favorable conditions. The profit in that case could reach 5.6-7.6 USD/m^3/crop (Chung and Sinh, 2011). While the model of medium-scale snakehead farming is more economical requirement and suitable for farmers with their own economic potential. Investment in such snakehead farming could result a farmer's livelihood improvement. The medium-scale model might bring the profit of 50-60 USD/m^3/crop (Thuy and Loc, 2015). Large-scale model (snakehead culture in pond) requires higher investment in financial terms than others model. However, such model could bring the highest profit among these models. According to Hien *et al*., (2012), snakehead pond culture had profit around 13,869 USD/ha/crop. Whereas Grimm-Greenblatt *et al*, (2015) indicated that small-scale farms using small-size fish (SSF) entirely had smaller fixed cost and greater variable cost than that of farms using pellet at the same scale. The high productivity farms (large-scale) using pellet had more beneficial than the farms using small-size fish (via the NPV and IRR indicators) and would continue to have higher economic benefit if moving to pellet. Low and medium productivity farms were more beneficial when using small-size fish (Grimm-Greenblatt et al, 2015). However, pond model has high risks and low profitability with only 37.5% of households being profitable (Navy *et al.,* 2016). Main difficulty of snakehead culture was dominant domestic consumption whereas finding stable markets internationally is a challenge. Snakehead price much depends on season, species and distribution channel (traders could put pressure on prices) (Chung and Sinh, 2011). Farmers lack capital for production while loans accounted for 50% of the capital requirements for production (Thuy, 2010). Accessibility to capital of farmers is also limited, this circumstance prevents residents take part in snakehead production (Loc, 2015). Development orientation of snakehead culture in Vietnam was proposed as below:

- Gradually reduce the amount of fish oil and fish meal in diets with soybean meal, rice bran or wheat bran (Navy *et al*., 2016).
- Replacing marine fish or golden snails in diets when freshwater small-size fish dropped (Thuy, 2010).
- To improve the quality of snakehead seeds, during nursing and breeding snakehead seeds, need to pay attention to sparse fingerlings in order to limit fish distribution that leads to eat together (Sang *et al.,* 2013).

CONCLUSIONS

In Cambodia, medium-scale snakehead culture system had the highest profit compared to small-scale culture system and also better than pond culture practice in both Cambodia and Vietnam. Cost and profitability of snakehead during 2006, 2011, and 2016 in Cambodia, indicated that the net profit of snakehead cage culture in 2006 was higher than the pond system, USD570 and USD 29, respectively (Table 2). Compared to the result in 2011, it shows that medium scale snakehead cage culture had highest profit (USD 4,353) compared to small scale. For large scale pond culture it was revealed that had profitability of USD383, whereas small scale and medium-scale farmers had negative profit (Table 3). Moreover, based on the results in 2016 it shows that the net profit was negative value for both snakehead cage and pond culture system, (-USD336) and (-USD11,491), respectively. This may be due to lifting of the ban on snakehead culture in Cambodia and have many snakehead farmers and surplus snakehead production. Similarly, the result was seen in Vietnam during the two-year study interval (2011 and 2016) that the bigger size pond culture system is the higher profitability system for farmers. The economic efficiency of pond culture system by production scale shows that small-scale pond culture farmers (<1000m2) received profit about 10 USD/m2/crop, while medium-and large-scale culture (>1000m2) received 60 USD/m2/crop.

In Vietnam, small-scale snakehead farming is properbly a feasible farming economic activity to the poor in rural area with the aim to improve nutrition and seafood consumption. Pond culture is a popular system for the households with financial potential in the form of high comercial industrial. Relevant stakeholders have stated that the quality of snakehead fingerlings tends to deteriorate. Therefore, consideration to the quality of broodstock in accompanied with investment in order to improve quality of seed is an urgent solution. Diseases in snakehead fish farming model appeared regularly together with incurable treatment. Selling prices fluctuated within year and drop significantly in 2016 and 2017. Stakeholders proposed that the public sector, especially government's support should take into account, especially export promotion to improve market prices.

It can be concluded that the major contributor to inputs cost for both cage and pond culture system in Cambodia and Vietnam derives from high feed cost. As clearly seen in the results of the study in Cambodia, the highest costs for snakehead cage and pond culture operation came from fish feed which contributes about 81% for cage and 75% for pond of total variable cost in 2006. Feed cost in 2011 makes up 88% and 49.37% for small- and medium- scale cage culture, respectively, and 53.48% for small, 69.54% for medium and 65.32% for large scale for pond culture. The share of variable cost from feed was still the largest cost in pond culture system which made up of 91% and 52%.

Therefore, it implies that the main factors for determining the profitability of snakehead aquaculture in both Cambodia and Vietnam are feed expenses and size of culture system operation. This call for attention from the relevant government institutions and agencies to provide support in terms of technical know-how and finance to develop/make pond and cage culture more productive and profitable and create employment opportunities for the rural poor. By promoting snakehead fish farming, it is hopefully expected that snakehead culture can, first, be used to sufficiently complement or replace wild snakehead fish which is decreasing, and, second, lower the price of fish to fit people's household income to guarantee that not only rich people but also the poor people can access to fish, particularly snakehead.

QUANTIFIABLE ANTICIPATED BENEFITS

The project has developed the best practice for snakehead culture in the pond model, which helps for snakehead farming to calculate their financial ability for investing. In addition, it helps increase economic efficiency and reduce risk in production.

The workshop training were carried out at An Giang, Tra Vinh provinces and final at Can Tho University for 141 scientists, researchers, resource managers, government officials, and non-government organizations and feed mill in Vietnam were better informed on the development of sustainable snakehead aquaculture through research based guidance on feeding, breeding, weaning and rearing/grow-out.

500 snakehead farming households in Cambodia will be better informed on the development of sustainable Giant snakehead aquaculture through research based guidance on feeding, breeding, weaning and rearing/grow-out.

Training on seed production, grow-out and fish diseases prevention and treatment for women at three provinces of An Giang, Dong Thap and Tra Vinh 106 participants in snakehead aquaculture in Cambodia and Vietnam will be better informed on the development of sustainable snakehead aquaculture through research based guidance on feeding, breeding, weaning and rearing/grow-out and on post-harvest activities including processing.

This investigation supported research activities of 1 master and dissertations of 2 undergraduate students (2 female).

Four research at CAF, CTU in Vietnam were trained and have experience on using economics to analyze specific impacts of cost and profitability of snakehead culture.

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TABLES AND FIGURES

Table 1. Number of participants in workshop-trainings of audience analysis in Cambodia

Stakeholders	BattamBang	Kampong Thom	National	Total
1. Farmers (small, medium, and large scale)	11	12	30	63
- Women	5	5	5	15
2. Private sectors	**8**	**10**	**18**	**36**
- Hatchery/nursing	0	0	1	1
- Feed and chemical company	2	2	2	6
- Processor	3	4	7	14
- Traders	3	4	8	15
3. Government officials	**12**	**12**	**25**	**49**
- Aquaculture + Fisheries Officers	2	2	5	9
- Researchers + Policy makers	10	10	15	35
- CTU-Vietnam	0	0	5	5
Total	**31**	**34**	**73**	**138**

Table 2. Cost and profitability of snakehead culture in cage and pond in 2006 in Cambodia.

Species: Giant Snaked Head	Total value per year/cycle (USD/Year)	
Items	Cage (n=9)	Pond (n=9)
I. Gross return	**3,588**	**356**
A. Total VC	2,748	270
B. Total FC	270	58
II. Total costs (TV+TF)	**3,018**	**327**
III. Gross profit (I - A)	**840**	**86**
IV. Net profit (I - II)	**570**	**29**
V. Real Profit (IV - Salary)	**225**	**-39**

Table 3. Cost and profitability of snakehead culture in cage and pond during year 2009- 2011 in Cambodia.

Species: Giant Snaked Head	Total value per cage (USD/Year)		Total value per pond (USD/Year)		
Items	Small (n=27)	Medium (n=15)	Small (n=24)	Medium (n=4)	Large (n=5)
I. Gross return	**1,746**	**7875**	**2,016**	**3,922**	**5,892**
A. Total VC	2,198	2,880	1,778	3,822	4,846
B. Total FC	295	642	392	171	663
II. Total costs (TV+TF)	**2,493**	**3,522**	**2,169**	**3,993**	**5,509**
III. Gross profit (I - A)	**-452**	**4,995**	**238**	**100**	**1,046**
IV. Net profit (I - II)	**-747**	**4,353**	**-153**	**-71**	**383**
V. Real Profit (IV - Salary)	**-835**	**3,959**	**-253**	**-267**	**89**

Table 4. Cost and profitability of snakehead culture in cage and pond during year 2016 in Cambodia.

Description	Total value per cage per year (USD/Year)	
	Cage (n=3)	Pond (n=2)
I. Gross return	**4,233.33**	**9,750.00**
A. Total VC	4,016.67	19,260.63
B. Total FC	552.36	1,980.83
II. Total costs (TV+TF)	4,569.03	21,241.46
III. Gross profit (I - A)	216.67	(9,510.63)
IV. Net profit (I - II)	(335.69)	(11,491.46)
V. Real Profit (IV - Salary)	(547.36)	(11,978.96)

Note: Fixed Cost (FC)

a. Depreciation of cage and materials/equipment:

- *Cage = (Purchasing Price) / (Number of expected years using) (Assuming that the Salvage value is equal to zero)*
- *Materials & Equipment= (Purchasing Price) / (Number of expected years using) (Assuming that the Salvage value is equal to zero)*

*c. Interest on borrowed funds = (Borrowed fund * Interest rate per year)*

Table 5. Number of participants in workshop-trainings of audience analysis in Vietnam.

Audience groups	An Giang	Tra Vinh	CTU	Total
1. Snakehead farmers	**22**	**20**	**26**	**68**
The poor	5	5	5	15
Women	14	7	10	31
2. Private sector	**8**	**5**	**8**	**21**
Seed production and nursing	2	1	2	5
Feed/drug traders	2	2	2	6
Processing	2	1	2	5
Fish Traders	2	1	2	5
3. Managers	**15**	**15**	**21**	**51**
Aquaculture managers	14	14	12	40
Political makers/researchers	1	1	6	8
NGOs			3	3
Total	**45**	**40**	**55**	**140**

Table 6. Cost – Profit analysis of snakehead culture small scale model (Hapas in river)

Indicators	Value (n=60)
Volume (m^3)	56.0
Stocking density (ind./m^3)	129
FCR	1.42
FCR small-size fish	*4.2*
Yield (kg/m^3/crop)	32.5
Production cost/crop (USD/m^3)	42.18
Selling price/kg (USD)	1.34-1.63
Production cost/kg (USD)	1.3
Revenue/Crop (USD/m^3)	43.48
Net profit/crop (USD/m^3)	1.29-10.9
Net profit/kg (USD)	0.04-0.34
Rate of return (%)	3.1-25.8

Table 7. Cost – Profit analysis of snakehead culture medium scale model (Hapas in pond)

Indicators	Value (n=30)
Volume (m^3)	61.5
Stocking density (ind./m^3)	94.2
FCR	1.32
Yield (kg/m^3/crop)	29.2-32.5
Production cost/crop (USD/m^3)	39.58
Selling price/kg (USD)	1.4-1.66
Production cost/kg (USD)	1.36
Revenue/Crop (USD/m^3)	41.0-53.81
Net profit/crop (USD/m^3)	1.42-9.76
Net profit/kg (USD)	0.05-0.3
Rate of return (%)	3.6-22.1

Table 8. Cost – Profit analysis of snakehead culture large scale model (culture in pond)

Indicators	**Value (n=70)**
Volume (m^3)	3,861
Stocking density (ind./m^3)	21.9-50
FCR	1.31
Yield (kg/m^3/crop)	16.2-30.0
Production cost/crop (USD/m^3)	20.81
Selling price/kg (USD)	1.20-1.30
Production cost/kg (USD)	1.28-1.32
Revenue/Crop (USD/m^3)	21.4-26.1
Net profit/crop (USD/m^3)	2.26-11.31
Net profit/kg (USD)	0.04-0.29
Rate of return (%)	2.7-21.0

Figure 1. Workshop – Training on snakehead culture development sustainability based on audience analysis in Inland fisheries research and development institute, Phnom Penh, Cambodia.

Figure 2. Group discussion in Inland fisheries research and development institute, Phnom Penh, Cambodia

Figure 3. Workshop-Training on snakehead culture development sustainability based on audiences analysis in Kompong Thom province, Cambodia

Figure 4. Workshop-Training on snakehead culture development sustainability based on audiences analysis in Battambang province, Cambodia

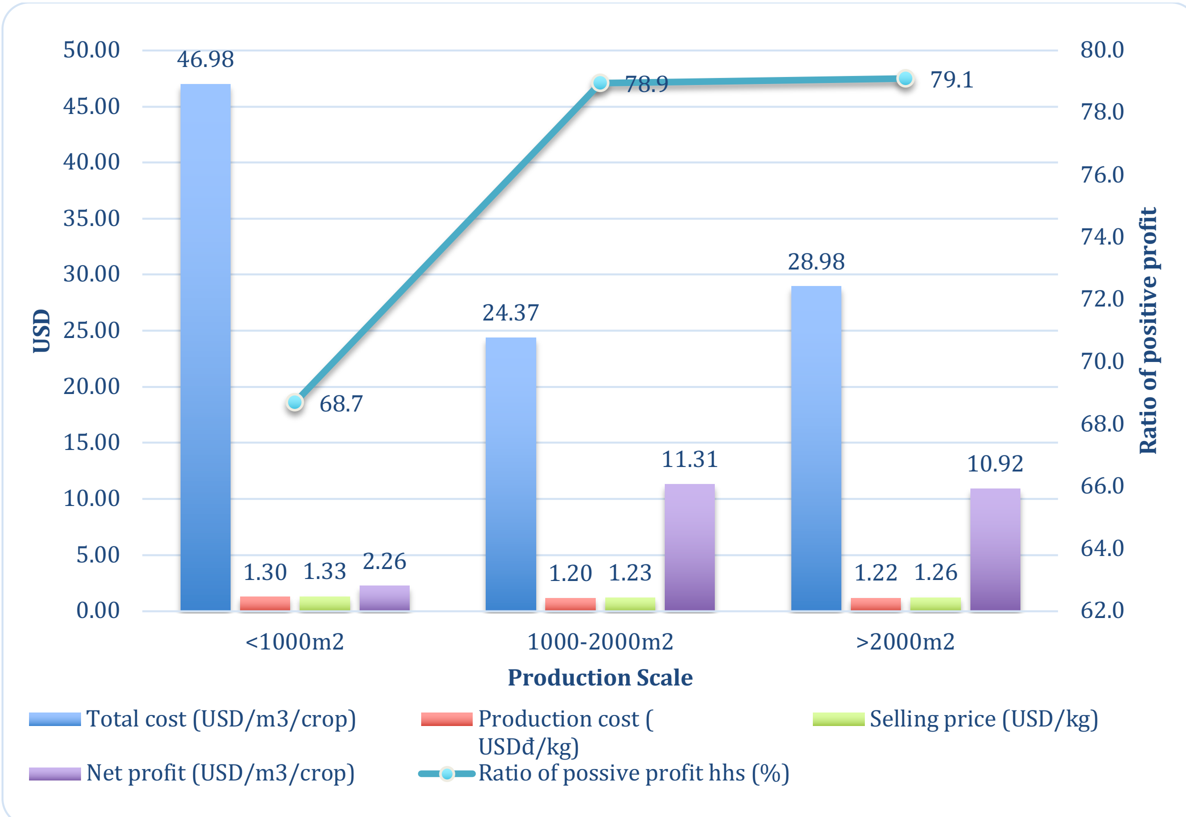

Figure 5. Financial efficiency by production scales in Vietnam.

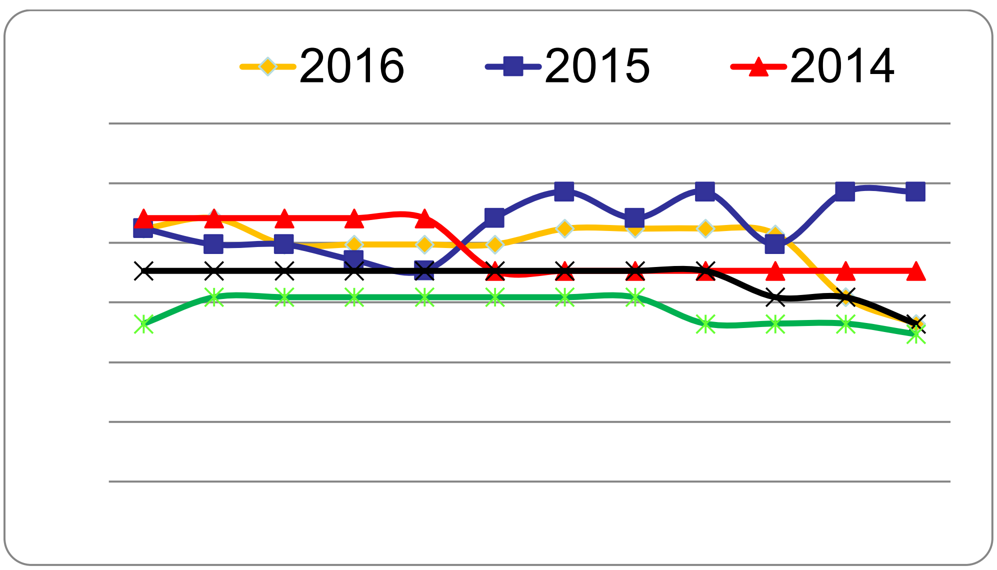

Figure 6. Selling price on market in Vietnam

Figure 7. Workshop – Training on snakehead culture development sustainability based on audiences analysis in An Giang prvince in Vietnam

Figure 8. Group discussion in An Giang province

Figure 9. Workshop – Training on snakehead culture development sustainability based on audiences analysis in Tra Vinh province


Figure 10. Group discussion in Tra Vinh province

Figure 11. Presentation result of group discussion in Tra Vinh province

Figure 12. Workshop – Training on snakehead culture development sustainability based on audiences analysis in Cantho University

Figure 13. Group discussion in Cantho University

Figure 14. Presentation in Cantho University

Figure 15. Participants attended on workshop in Cantho University

TOPIC AREA

MARKETING, ECONOMIC RISK ASSESSMENT, AND TRADE

❖

ENHANCING THE FUNCTIONALITY AND APPLICABILITY OF FISH MARKET INFORMATION SYSTEM (FMIS) TO MARINE ARTISANAL FISHERIES IN GHANA

Marketing, Economic Risk Assessment, and Trade/Activity/16MER01PU

Kwamena Quagrainie[1], Stephen Amisah[2], and Alloysius Attah[3]

[1]*Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA*
[2] *Kwame Nkrumah University of Science & Technology, Ghana*
[3]*FarmerLine, Ayeduase Street, Kumasi, Ghana.*

ABSTRACT

This investigation expanded the functionality of the Fish Market Information System (FMIS) funded during the AquaFish Innovation Lab 2013-2015 funding cycle that developed a pilot cell-phone based FMIS with a focus on farmed tilapia and catfish in Ghana. The enhancement included prices of marine species at selected landing sites, and access of the system to consumers. The species included are: (1) Red fish (*Dentex angolensis*) locally referred to as "Wiriwiri." (2) *Caranx* species (locally called "kpanla"), (3) Mackerel (*Scomber sp*), wrongly but locally referred to as salmon), (4) Tuna (*Tunus tunus*), and (5) *Sardinella* species (also called Herring) – the two common species are *Sardinella aurita* and *sardinella eba*. By providing fishermen easy access to price information, and fish mongers with information on where to buy fish further empowers these value chain agents to operate efficiently. Also, allowing consumers of fish access to market prices further closes the gap between what farmers / fishermen are making and what consumers are paying. The enhanced FMIS thus created an enabling environment where stakeholders in the fish value chain are better informed. The improved system helps fish farmers/fishers, fish processors and traders to more efficiently support urban markets with seafood products. In addition, the improved FMIS has applicability to the marine artisanal fisheries subsector from fish trade.

The enhanced FMIS delivers information in 5 languages – English, Twi, Ga, Ewe and Fante. For SMS/access code, SMS/text to the FMIS by dialing *399*23# on a local Ghana phone number. The FMIS has an online database that can be accessed with registration at https://mis.mergdata.com. Once registered, access to the fish information is at https://core.mergdata.com.

Two training workshops were organized for artisanal fishermen and fish mongers on the use of the technology. The first workshop was held in Elmina in the Central region on May 16, 2017 in the Conference Hall of the District Assembly, Komenda-Edina-Eguafo Abrem (KEEA), off the main Cape Coast-Takoradi road. There were a total of 55 participants, which included 38 males and 17 females. Participants included fishermen, canoe operators, community leaders, fish mongers, municipal officials, and scientists from the University of Cape Coast. The second workshop was held at Bortianor in the Greater Accra region on May 23, 2017. This was also a one day workshop, and there were a total of 48 participants, which included 28 males and 20 females.

INTRODUCTION

An analysis of tilapia value chain in Ghana under a previous AquaFish CRSP project revealed challenges in the flow of information along the value chain, especially information relating to tilapia supply, demand and prices. For small-scale fish producers and artisanal fishermen, readily available market information on prices and demand for fish at different fish markets will help inform production and harvesting decisions. Minimizing the information gaps along the fish value chain will greatly improve efficiencies in fish marketing and the value chain as a whole. There was a need therefore to develop a marketing information system for tilapia in Ghana. Consequently, the AquaFish Innovation Lab funded an investigation during the 2013-2015 funding cycle that developed a cell-phone based Fish Market Information System (FMIS) with a focus on tilapia and catfish in Ghana. Tilapia and catfish are the dominant farmed fish species that are also caught in inland waters. The FMIS is web-based and provides market information on the two species on-line as well as via voice and SMS/text messaging to users. It has a database of farm-gate and market prices of the two species in selected locations in Ghana assembled by fisheries officers and selected agents.

There are two types of subscribers to the system – registered users and ad-hoc users. The system can send out (push) farm-gate and market price information to only the registered users. However, to request (pull) information on tilapia prices from the system, both registered users and ad-hoc users can access the system by dialing or SMS/text messaging to a 10-digit phone number or a 4-digit short code. The voice feature of the system when a user requests for information includes messages in English and three native languages – Twi, Ga and Ewe.

This investigation expanded the functionality of the current FMIS with more value chain services to include prices of marine species at selected landing sites, add another local language (Fante) and access of the system to consumers. The artisanal fishery plays an important role to coastal communities by providing employment, revenue, and a resource for food. It contributes to the national economy in terms of food security, employment, poverty reduction, GDP and foreign exchange earnings. However, the artisanal fisheries are confronted with challenges, which include high post-harvest losses and handling costs as well as low economic returns and low value addition (Aheto et al., 2012; Mills et al., 2012; Mensah & Antwi, 2002). Artisanal fishers depend on inland and marine resources and their contribution to the national food system required appropriate investments in developing the seafood value chain to reduce the waste, enhance efficiency, and strengthen value addition. Therefore, the applicability of the existing FMIS was broadened to include the marine artisanal fisheries subsector, which goes a long way to improving the welfare of artisanal fishermen through a reduction in transaction costs and improvement in the benefits from fish trade.

Allowing consumers of fish access to market prices via the FMIS further closes the gap between what farmers and fishermen are making and what consumers are paying. The FMIS thus creates an enabling environment where stakeholders in the fish value chain are better informed. The improved system helps fish farmers/fishers, fish processors and traders to more efficiently support urban markets with seafood products.

OBJECTIVES

1. Broaden the applicability of existing FMIS to include the marine artisanal fisheries subsector.
2. Expand the functionality of the FMIS by customizing market price collection procedures to enable accurate and real time data collection.
3. Train marine artisanal fishermen on the use of the FMIS.

METHOD

Objectives:

1. Broaden the applicability of existing FMIS to include the marine artisanal fisheries subsector.
2. Expand the functionality of the FMIS by customizing market price collection procedure to enable accurate and real time data collection.

Farmerline provided additional programming that broadened the applicability which allows marine artisanal fishermen to obtain market prices for 5 major marine species landed and found in major markets via voice and SMS/text messaging. The species are: (1) Red fish (*Dentex angolensis*) locally referred to as "Wiriwiri." (2) *Caranx* species (locally called "kpanla"), (3) Mackerel (*Scomber sp*) (wrongly but locally referred to as salmon), (4) Tuna (*Tunus tunus*), and (5) *Sardinella* species (also called Herring) – the two common species are *Sardinella aurita* and *sardinella eba*.

Artisanal fishermen are able registered with the system and requested market information through a short code. Fisheries officers and agents were recruited to visit selected landing sites along the coastal regions of Ghana as well as major retail markets to obtain prices. Discussions were held with the Ministry of Fisheries and Aquaculture Development (MFAD) on providing assistance through the fisheries officers. FarmerLine's field officers assisted with the collection of market prices. A weighted average weekly price were calculated and made available to users.

The improved FMIS enhanced the quality and timeliness of data collected through customization of the MERGDATA platform. The data collection process was complemented with crowd-sourced information from consumers selected randomly to provide vital feedback on the validity of the prices being received. New partnerships were developed with the marine sector, which expanded the scope of engagement with information assembled in the FMIS database. The enhanced FMIS provides more accurate and timely market information. The languages of delivery are English, Twi, Ga, Ewe and Fante. The messages are sent periodically by 8 agents in Weija, Apam and Elmina from landing beaches in the Central region using the USSD code assigned to the FMIS, *399*23*1#.

Objective:

3. Train marine artisanal fishermen on the use of the FMIS.

Two training workshops were organized for artisanal fishermen and fish mongers on the use of the technology to access information to facilitate their business transactions relating to fish availability, sales and sales points, and prevailing prices. The workshops took place in Elmina in the Central region, and Bortianor in the Greater Accra region, both vibrant and historic fishing towns along the coast. The training activities were conducted in collaboration with the MFAD, chief fishermen, and elders in the selected fishing communities. Regional and District fisheries officers participated in the training of the artisanal fishermen.

RESULTS

Objectives

1. Broaden the applicability of existing FMIS to include the marine artisanal fisheries subsector.
2. Expand the functionality of the FMIS by customizing market price collection procedure to enable accurate and real time data collection.

A summary of results from Farmerline is presented in Figure 1. FarmerLine delivered the first fish market information using the FMIS to 23 fishermen and fish traders who had registered to receive the weekly prices. The traders were all women. Three hundred and sixty-seven (367) fish price messages were sent; 308 via SMS and 59 via voice calls. All subscribers successfully received the messages.

Forty-two (42) out of the 59 calls were answered and listened to in full by the farmers, thus the message completion rate was 100%.

Farmerline successfully expanded the FMIS platform for some marine fish species as a way of enhancing efficiency of market information through mobile messaging. This system has provided a direct relationship between the fish product and fishermen, and bridged the information gap for tilapia, catfish from the previous AquaFish FMIS and marine species (Redfish – *Dentex Angolases*, Herrings, *Sardinella spp*, Tuna, *Carans spp* and Mackerel fish) for fish farmers and fishermen.

At the completion of the project, 12 fishermen of the total who had registered and were receiving messages were picked at random, and their views sampled on various aspects of the prices they received. Approximately 92% were happy with the clarity of the messages on prices.

The FMIS has a database of fish quantities, prices and other market information and a platform for sharing market information. For online access to the database of fish prices, registration is required. Visit: https://mis.mergdata.com. Once registered, access to the fish information is at https://core.mergdata.com. For SMS/access code SMS or text to the FMIS by dialing *399*23# on a local Ghana phone number.

Objective:

3. Train marine artisanal fishermen on the use of the FMIS.

The workshop in Elmina in the Central region was held on May 16, 2017 in the Conference Hall of the District Assembly, Komenda-Edina-Eguafo Abrem (KEEA), off the main Cape Coast-Takoradi road. There were a total of 55 participants, which included 38 males and 17 females. Participants included fishermen, canoe operators, community leaders, fish mongers, municipal officials, and scientists from the University of Cape Coast (UCC). The scientists, professors, and students from UCC, and the Regional Fisheries Officer who attended the workshop found the initiative exciting and very relevant to the fishermen they worked closely with. In Ghana, women are not allowed to go fishing but most often, they exclusively handle the fish after landing by the male fishermen.

The meeting began with an outline of what USAID AquaFish Innovation Lab has accomplished over the years in Ghana with fish farming and the purpose of the FMIS technology. Mr. Amos Wussah from FarmerLine went through in detail the functionality of the FMIS and the importance to participants. Participants received various short codes from FarmerLine to access the database to find out fish type, location, prices, language in which they want to communicate, and check for relevant market information using their own cell phones, which everyone had. There were very lively discussions on the FMIS and some of the issues that arose included:

1. Participants provided indigenous names for 5 common marine species but the names appeared to vary by locations along the coast. Other common species were suggested to be added to the list to populate the FMIS platform.
2. Suggestions were also made to extend the FMIS training to nearby coastal fishing communities such as Komenda, which was said to host many more artisanal fishers because it is a conglomerate of 4 fishing communities.
3. Participants were excited about the FMIS and grateful for the initiative by AquaFish Innovation Lab and asked for a continuous refresher training from time to time.

The second workshop was held at Bortianor in the Greater Accra region on May 23, 2017. This was also a one-day workshop. There was a total of 48 participants, which included 28 males and 20 females. Similar issues encountered in the Elmina workshop were also brought up during the

Bortianor workshop. The participants provided the indigenous names to the common marine species and would like to see other common fish species added to the database.

CONCLUSIONS

Overall, the FMIS has been a success for a pilot program. Some of the challenges encountered are the absence of clear standards for fish products and pricing, buy-in from all stakeholders, which is important for long term sustainability of the FMIS on a pay-per-use basis, and publicity of the tool. There are diverse clientele and information needs of different stakeholders (e.g., input suppliers, fish farmers, fish traders, policy makers, consumers, etc), which suggests further scalability – expansion to attract more users moving forward. Long-term sustainability of the FMIS requires a pay-per-use system and arrangements on revenue sharing with mobile phone service providers. FarmerLine has added FMIS as an integral part of their portfolio of services to increase the benefits to users.

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Mensah, J.V., & Antwi, B.K. (2002). Problems of Artisanal Marine Fishermen in Ghana: The Way Ahead. Singapore Journal of Tropical Geography, 23(2); 217-235.

Mills, D.J., Mutimukuru-Maravanyika, T., Ameyaw, G., & Asare, C. (2012). Ghana Coastal Fisheries Governance Dialogue: Presentations, discussions and outcomes from a stakeholder forum on issues for reforming governance of Ghana's coastal fisheries. WorldFish Center, USAID Hɛn Mpoano Initiative, Ghana. 57pp. Available at: http://www.worldfishcenter.org/resource_centre/WF_3450.pdf

TABLES AND FIGURES

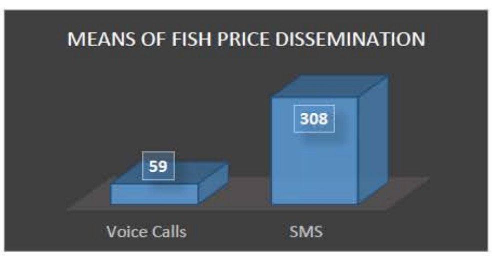
Farmerline

FISH MARKET INFORMATION SYSTEMS

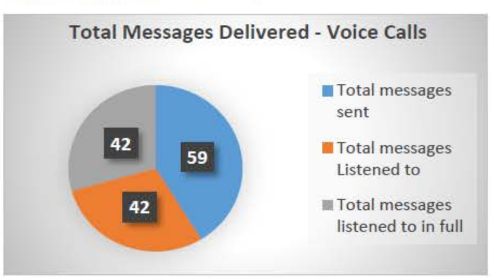
USAID Aquafish Ghana Innovation Lab, KNUST

Farmerline has developed and implemented an extraordinarily successful Market Information System platform for some fresh water and Marine fishes in improving the profits of farmers by enhancing the efficiency of input use and increasing the size of the average fish by delivering concrete suggestions and market information through mobile messaging.
By providing a direct relationship between product and farmers, the Aquafish MIS project has closed the information gap for farmers of tilapia, catfish and marine fishes.

Content delivery started on May 02, 2016 to twenty-three (23) farmers and women who trade in fish, and has registered to receive the weekly prices. A total of **367 fish price messages delivered in SMS (308) and Voice Calls (59)**. All the 308 SMS were received by the subscribers.



Out of the 59 fish prices that were sent through voice calls, a total of forty-two (42) calls were picked up (answered) and listened to by the farmers. **The call pick-up rate on the first ring averaged 71.2%.** The farmers who picked up the calls listened to the messages in full (42), thus, **the message completion rate was 100.00%.**

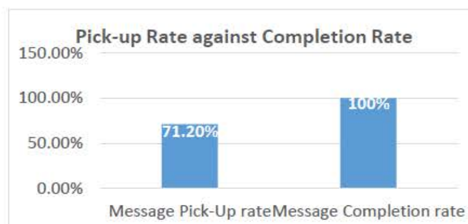


Project Duration:
2nd Febuary, 2017 – 30th May, 2017

Implementing Partners:
USAID Aquafish Ghana Innovation Lab

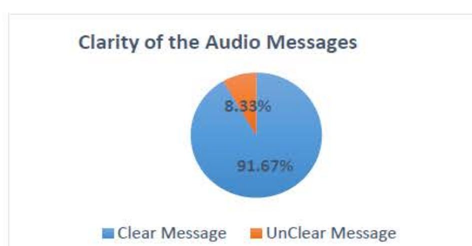
Targeted Fish Species (7):
Fresh water: Tilapia & Catfish
Marine: Red fish - Dentex Angolases, Herrings (Amane) - Sadinalla species, Tuna, Carans (Kpala) & Mackerel (Salmon)

Languages for Content Delivery:
Twi, English, Ga, Ewe, Fante



Eight (8) agents from Weija, Apam and Elimna periodically send fish prices from the landing beaches using the USSD code assigned to the FMIS, *399* 23*1#.

At the end of the project, twelve (12) farmers (52% of the farmers who re) out of the total who registered and are receiving fish prices were picked at random to sample their view on various aspect of the prices they received. According to the responses, **91.67% were happy with the clarity of the messages with prices.**



www.farmerline.co

MIS to USAID AQUAFISH INNOVATION LAB FACTSHEET

Figure 1. Farmerline summary of results.

ASSESSMENT OF PRICE VOLATILITY IN THE FISH SUPPLY CHAIN IN UGANDA

Marketing, Economic Risk Assessment, and Trade/Activity/16MER02AU

James O. Bukenya

Alabama A&M University, 4900 Meridian Street, Normal, AL 35762

The results for this investigation were published in two peer-reviewed journal articles. The full citations, links, and abstracts are included below.

Bukenya, J. O. (2017). Assessment of Price Volatility in the Fisheries Sector in Uganda. Journal of Food Distribution Research, 48(1), 81–88.
Publication link: https://www.fdrsinc.org/wp-content/uploads/2017/03/JFDR_48.1_18_Bukenya.pdf

ABSTRACT

This paper examines price volatility in the African catfish (*Clarias gariepinus*) supply chain in Uganda. The volatility process in the catfish markets was analyzed based on monthly price data from January 2006 to August 2013. A GARCH model is used to estimate the volatility parameters. Empirical results revealed that the value of the first-order autoregressive term and the value of the first-order moving average term were significant for both aquaculture and wildharvest catfish supply chains. The observed long persistence of volatility in both supply channels suggests a fundamental level of uncertainty and risk in the catfish subsector over the studied period.

Bukenya, J. O. (2017). Forecasting Farm-Gate Catfish Prices in Uganda Using SARIMA Model. Finance and Market, 2(2), 1–12. http://doi.org/10.18686/fm.v2i2.1047
Publication link: http://ojs.usp-pl.com/index.php/fm/article/view/1047/969

ABSTRACT

Stabilization of prices of essential agricultural commodities continues to remain an area of major concern for policy makers; given that price instability affects both producers and consumers, and has macroeconomic implications. This paper examines farm-gate price behavior in the African catfish markets in Uganda, and develops a forecasting model that adjusts for the seasonal fluctuations in the price series. The analysis utilizes monthly catfish real price series for the period January 2006 to December 2013. The model provides good in-sample and out-of-sample forecasts for the eight-year time period. The out-sample predictions based on SARIMA (1, 1, 1) (0, 1, 1)12 model suggest that the stochastic seasonal fluctuations depicted in the price series are successfully modeled, and that catfish real prices follow an upward trend. The findings can assist policy makers and major stakeholders to gain insight into more appropriate economic and sectorial policies that can lead to the development of reliable market information systems and up-to-date data on catfish supply, demand and stocks.

TOPIC AREA

MITIGATING NEGATIVE ENVIRONMENTAL IMPACTS

❖

ADVANCING SEMI-INTENSIVE POLYCULTURE OF INDIGENOUS AIR-BREATHING FISHES, KOI AND SHING, WITH MAJOR INDIAN CARPS FOR ENHANCING INCOMES AND DIETARY NUTRITION WHILE REDUCING ENVIRONMENTAL IMPACTS

Mitigating Negative Environmental Impacts/Experiment/16MNE01NC

Shahroz Mahean Haque[1], Joythi Saha[1], Tahmin Toma[1], Fariha Reza[1], Ratumoni Romana[1], Courtney A. Deck[2], and Russell J. Borski[2]

[1]Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA

ABSTRACT

The use of Koi or climbing perch (*Anabas testudineus)* in aquaculture has grown substantially over the past decade in Bangladesh because it has a high market value and is rich in nutrients. Further, being an air-breathing fish, Koi have a strong capacity to tolerate poor oxygen environments. Koi production is currently limited to monoculture systems with intensive use of commercial-grade feeds. Feed constitutes almost 80% of the total costs for producing Koi and thus methods to reduce feed inputs can provide significant economic benefits, particularly if fish are co-cultured with carps that rely primarily on natural pond productivity rather than direct consumption of formulated feeds. Thus, the aim of our first study was to investigate growth and production of Koi when used in polyculture with major Indian carp species, Rui (*Labeo rohita*) and *Catla* (*Catla catla*) relative to that observed with Koi monoculture. We also examined the effects of combining reduced feed ration and pond fertilization on Koi-carp polyculture. The experiment consisted of four treatments, with three replicates each (12 ponds; 100 m^2 area, 1.5 m depth). T1 consisted of a Koi monoculture ($5/m^2$) with full daily feeding while the other three treatments consisted of a Koi-carp polyculture (Catla, $0.2/m^2$; Rui, $0.8/m^2$; Koi, $5/m^2$) with full daily feeding (T2), 75% daily feeding (T3), or 50% daily feeding (T4). Additionally, the ponds for T3 and T4 were fertilized weekly with urea and triple super phosphate (28 kg/ha N, 5.6 kg P/ha) to boost pond productivity. Koi were fed a full daily ration of commercial feed (CP feed) according to current practice (20% down to 5% body weight/day) or a fraction of this based on treatment groups. Growth and production of Koi was greater in polyculture with carps (T2-4) than in monoculture and production increased with decreasing feed ration. Thus, the highest production of Koi was in T4 (3484 kg/ha) and the lowest production was in T1 (1963 kg/ha). No significant differences were observed in growth or production of Rohu, although both were slightly higher in T4, while Catla production was significantly lower in T3 (393 kg/ha) relative to T2 (716 kg/ha) and T4 (634 kg/ha) likely due to the lower specific growth rates and survival rates for T3. Total production, net return, and benefit cost ratio were all greatest in T4, which employed a 50% reduction in feed, and then decreased with increasing feed ration. Overall, combining the culture of Koi with carps is more economical and can increase production and earnings for fish farmers in Bangladesh. Additionally, reducing the amount of feed applied to the ponds by 50% mitigates production costs and enhances feed conversion which can further increase food availability and incomes for rural farming households.

The stinging catfish or shing (*Heteropneuestes fossilis*) is another high value, micronutrient dense, air-breathing fish that has a strong capacity to tolerate poor oxygen environments and thus culture of Shing has also been increasing in Bangladesh. In a second study, we investigated the effects of combining Shing at different stocking densities into Koi-carp polyculture. This study consisted of four treatments with three replications. T1 consisted of a Koi-carp polyculture without Shing (Catla, $0.2/m^2$; Rohu, $0.8/m^2$; Koi, $5/m^2$), while T2-4 contained Shing stocked at the densities of $1.0/m^2$, $2.0/m^2$, and $3.0/m^2$, respectively. A reduced 50% feed ration (10% down to 1.5% body weight/day) was applied to the ponds based on the biomass of both Koi and Shing which was adjusted every two weeks from fortnightly sampling of fish weights and all ponds were fertilized weekly. Production and survival of Koi, Rohu, and Catla was higher in ponds containing lower stocking densities of Shing (T2 and T3). Gross production and returns were greatest in T4 because of the increased abundance and higher market value of Shing. However, due to the higher cost of feed and lower survival rates for all species in T4, the greatest net profit and benefit cost ratio was observed in T3 indicating that incorporating Shing into Koi-carp polyculture at a stocking density of $2.0/m^2$ would be the most beneficial for increasing food production and incomes for rural farmers in Bangladesh.

INTRODUCTION

Air-breathing fishes provide a significant advantage for pond culture, as they tend to be resilient to harsh conditions, particularly during periods of low-oxygen, which can occur with high temperatures, drought, or poor water quality. Indigenous air-breathing fishes, such as Shing catfish (stinging catfish, *Heteropneustes fossilis*) and Koi (climbing perch, *Anabas testudineus*) are commonly found in open waters, paddy fields, and swamps of Bangladesh. Because of accessory respiratory organs they can even survive for a few hours out of the water. These fishes have been successfully cultivated in Bangladesh in recent years and command a high market value (DOF, 2012; Kohinoor et al., 2011), 3-7 times that of other commonly cultured finfishes (striped catfish or *Pangasius* and tilapia). Both are currently in great demand by consumers for their taste and nutritional value (Hasan et al., 2007, Vadra, 2012; Vadra and Sultana, 2012). Shing catfish is particularly high in both iron (226 mg 100 g^{-1}) and calcium relative to other freshwater fishes and has been recommended in the diets of the sick and convalescent (Saha and Guha, 1939; Singh and Goswami,1989). Culture of these indigenous species with high mineral content is an important step for increasing the yield and diversity of aquaculture products for consumption in Bangladesh and in reducing some types of dietary malnutrition, such as iron-deficient anemia (Dey et al., 2008; Micronutrient Initiative/UNICEF, 2004).

Production of Shing and Koi is currently limited to monoculture systems with high stocking densities and intensive use of commercial-grade feeds (30-35% crude protein). As feed can comprise a majority of total production costs (> 70%), there is limited participation by small homesteads utilizing the current practices for these fish and thus creating a significant impediment to further expansion of this industry. Further, the use of high-levels of feed inputs has led to a persistent deterioration of pond water quality (eutrophication; cf. Chakraborty and Mirza, 2008; Chakraborty and Nur, 2012) and periodic mass mortalities and disease outbreaks. As most ponds are located near homesteads and villages, poor water quality and foul odors related to greater nutrient-loading impacts both local health and socio-economic tensions within the community (personal communication, Nural Amin, local farmer in Tarakanda, Mymensigh, July, 2012). Through field visits to Mymensingh, this research team observed firsthand that most air-breathing fish farms are often overfed, thus some of the problems associated with farming of air-breathing fishes can be alleviated through better management and implementation of semi-intensive culture practices. These problems may also be mitigated through polyculture, where excess nutrients and algae can be utilized by other species, for instance carps that feed primarily on plankton.

To this end, in Phase I of our project we evaluated whether carps could be incorporated into pond culture of Shing catfish. We found that addition of indigenous Indian carps (Rohu and *Catla*) enhances total fish yields and nutrient utilization of feed inputs over that seen with Shing monoculture alone. Shing growth was little impacted by culture with carps. Moreover, we found that reducing ration levels by as much as 50% from those currently used by the farming community (*e.g.* 20-5% body weight/day) provides additional return on investment of almost 100% in Shing-carp polyculture. We also demonstrated that Koi could be successfully cultured with either *Catla* alone or with *Catla* and Rohu under the reduced feeding ration established for Shing. However, our studies did not compare Koi-carp polyculture with Koi monoculture or whether the 50% reduction in feed inputs utilized produces similar growth and fish yields as could be seen with feeding at a higher rate. Therefore, we first assessed whether mixed trophic polyculture of Koi and carps is a better technology than Koi monoculture and whether feed reductions can produce equivalent or better production yields and can improve nutrient utilization and water quality over current feeding practices.

Recent studies using high stocking densities (25-37 fish/m^2) and prohibitively high feed inputs (100% down to 5% body weight/day) suggests that both Shing and Koi can be cultured together (Chakraborty and Nur, 2012). We propose to extend the new semi-intensive Koi-carp technology developed here to evaluate whether Shing might provide additional increases in fish yields and returns on investment in Koi-carp polyculture. Indeed, farmers are now interested in understanding if culture of both air-breathing fishes with carps might provide economic advantages, particularly under a reduced feed ration. Here we assessed the addition of Shing stocked at different densities in Koi-carp growout. To our knowledge, the incorporation of Shing, Koi, and carps in polyculture has yet to be evaluated and this could represent an additional technology for enhancing efficiency of food production in ponds, yield of nutritious fish, and farmer incomes.

OBJECTIVES

1. Compare combined polyculture of Koi with two major carps (Rohu and *Catla*) versus Koi monoculture under semi-intensive pond culture conditions.
2. Assess the effect of reduced feed ration in polyculture of carps and Koi. This study will identify a feed-reduction ration needed for equivalent or better production yields through increased nutrient utilization efficiency and impacts on the environmental water quality.
3. Assess economic and environmental benefits of combining Shing with Koi-carp pond polyculture.
4. Evaluate overall performance and economic returns of the improved management strategies.

MATERIALS AND METHODS

Location

These studies was performed onsite at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Water quality analysis was performed at the Water Quality and Pond Dynamics Laboratory (BAU).

Pond Preparation

Prior to initiating each study, the ponds at the BAU Fisheries Field Laboratory (100 m^2, 1.5 m depth) were dried, re-excavated, and limed (25 g $CaCO_3$/m^2). Ponds were then fertilized with 28 kg N/ha as urea and 5.6 kg P/ha as triple super phosphate (TSP) prior to being stocked with the appropriate species.

Study 1- Assess reduced ration levels for combined polyculture of two major carps (Rohu and Catla) with Koi

This study evaluated the effects of 75% and 50% daily rations on growth, production yield, and economic returns for semi-intensive Koi-carp polyculture. Four different treatments were evaluated as outlined in Table 1 and each treatment was replicated in 3 separate ponds. The initial stocking weights were 0.69 ± 0.08 g, 21.76 ± 7.76 g, and 32.27 g for Koi, Rohu, and *Catla*, respectively. During the experimental period, ponds belonging to T3 and T4 were fertilized weekly at a rate of 28 kg N/ha (urea) and 5.6 kg P/ha (TSP) while T1 and T2 did not receive any fertilizer treatments. Ponds in T1 and T2 were given full daily rations of a floating commercial feed (30% crude protein) at the rates currently employed by farmers (20% bw/day, 0-30 days; 15% bw/day, 31-60 days; 10% bw/day, 61-90 days; 5% bw/day, > 90 days) while T3 ponds received 75% daily rations and T4 received 50% daily rations. The percentage of feed applied to each pond was based on the biomass of Koi alone. Water quality parameters (temperature, transparency, etc.) were measured fortnightly while plankton and benthos samples were collected fortnightly and monthly, respectively. All ponds were sub-sampled every 15 days for growth measurements and upon study completion or after 126 days the specific growth rate (SGR) for each species was calculated. Total feed conversion ratios (FCR) and cost-benefit analyses were also calculated at the end of the study.

Table 1. Experimental design for Study 1.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Rohu (*L. rohita*)	0	80 (0.8/m^2)	80 (0.8/m^2)	80 (0.8/m^2)
Catla (*C. catla*)	0	20 (0.2/m^2)	20 (0.2/m^2)	20 (0.2/m^2)
Koi (*A. testudineus*)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)
Fertilization (/ha)	0	0	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P
Daily Feeding	100% Ration	100% Ration	75% Ration	50% Ration
Replicates (*n*)	3	3	3	3

Study 2- Effect of combining Shing at different stocking densities in Koi-carp polyculture

This study assessed whether Shing catfish could be incorporated into Koi-carp polyculture to provide an additional crop of high nutritional and economic value for farmers. Three different stocking densities of Shing were evaluated (T2- 1.0/m^2, T3- 2.0/m^2, T4- 3.0/m^2) and compared to a control Koi-carp culture with no Shing (T1, Table 2). Koi, Rohu, and *Catla* were stocked at the same densities from Study 1. During the experimental period, ponds were fertilized at a rate of 28 kg N/ha/week (urea) and 5.6 kg P/ha/week (TSP) and each pond received a 50% daily feed ration (10% based on the biomass of Koi and Shing in each treatment. Water quality parameters (dissolved oxygen, pH temperature, transparency, ammonia, nitrates, nitrites, phosphates, alkalinity, and chlorophyll-a) were measured fortnightly. All ponds were sub-sampled every 15 days for the collection of growth data. Upon conclusion on the study at 140 days, SGR, FCR, and production yields were calculated and a cost-benefit analysis performed.

Table 2. Experimental design for Study 2.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Rohu (*L. rohita*)	80 (0.8/m^2)	80 (0.8/m^2)	80 (0.8/m^2)	80 (0.8/m^2)
Catla (*C. catla*)	20 (0.2/m^2)	20 (0.2/m^2)	20 (0.2/m^2)	20 (0.2/m^2)
Koi (*A. testudineus*)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)
Shing (*H. fossilis*)	0	100 (1.0/m^2)	200 (2.0/m^2)	300 (3.0/m^2)
Fertilization (/ha)	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P
Daily Feeding	50% Ration	50% Ration	50% Ration	50% Ration
Replicates (*n*)	3	3	3	3

Equations
SGR (% bw d^{-1}) = [{Ln (final weight) – Ln (initial weight)} ÷ Culture period in days] × 100
Survival (%) = (Number of fish harvested ÷ Number of fish stocked) ×100
Gross production = Number of fish harvested × Final weight of fish
FCR = Amount of feed applied to pond ÷ Total fish weight gain

Statistical Analysis
All growth and economic parameters were analysed with a one-way ANOVA and Tukey's HSD post-hoc test (JMP 13).

RESULTS AND DISCUSSION

Study 1- Assess reduced ration levels for combined polyculture of two major carps (Rohu and Catla) with Koi

This experiment consisted of four treatments that aimed to determine whether reduced feeding rations can be successfully applied to Koi-carp polyculture. All water quality parameters (measured fortnightly) were deemed suitable for fish culture throughout the study period and did not differ significantly between treatments (Table 3). We identified 28 genera of phytoplankton and 12 of zooplankton over the course of the study period in all ponds, however no differences in total plankton abundance were observed (Table 4). In addition to plankton, we measured the abundance of benthic organisms in each treatment as these can also serve as a food source for carps. Species were grouped into four classifications (oligochaeta, chironomidae, Mollusca, or unidentified) and total abundance was also calculated. Although not statistically significant, the Koi monoculture (T1) had a greater overall abundance of benthic organisms relative to the combined Koi-carp cultures, likely due to the consumption of these organisms by the two carp species. Further, the abundance had a tendency to decrease with decreasing feed ration suggesting that the fish may be increasing their consumption of natural food sources within the ponds to compensate for the reductions in commercial feed.

Although there were no significant differences in growth or production between treatments for Rohu, production was slightly higher in T4 which provided the 50% reduced feeding ration (Table 5). Catla production was significantly lower in T3 (393.0±33.8 kg/ha) relative to T2 (715.5±32.8 kg/ha) and T4 (634.1±26.4 kg/ha) which is likely due to both the lower specific growth rates and the lower survival rates in this group (Table 5). Koi growth and production was higher in polyculture (T2-4) than in the monoculture (T1, 1963.2±5.87 kg/ha) and tended to increase with decreasing food ration (Table 5). Thus, production was greatest in T4 (3484.0±37.8 kg/ha), followed by T3 (3311.0±215 kg/ha) and T2 (2311.6±38.4 kg/ha). T4 also exhibited the highest total production for all species and the best feed conversion ratio (Table 5), indicating that current guidelines overestimate the amount of feed required and a 50% reduction combined with pond fertilization to increase natural productivity could reduce the costs of fish culture. Indeed, T4 provided the greatest net return (540,772±28185 BDT/ha) and benefit cost ratio (2.18) relative to all other treatments (Table 6). The second highest return was in T3 (400,122±43361 BDT/ha), followed by T2 (266,644±12670 BDT/ha), and T1 (76,051±1056 BDT/ha). Our results indicate that the addition of Koi to carp polyculture enhances production of this species which is beneficial for the fish farmers as Koi fetch a higher market value than carps. Further, we have shown that reducing feed rations by 50% in Koi-carp polyculture enhances feed conversion ratios, production, and profits. Thus, adopting these practices including feeding at a rate range of 10%-2.5% body weight/day could increase fish production, food availability and incomes for rural fish farmers in Bangladesh.

Study 2. Effect of combining Shing at different stocking densities in Koi-carp polyculture
This study assessed the impact of adding Shing at different densities to Koi-Carp polyculture. Ponds were fertilized throughout the study to provide primary productivity for carps, while a 50% feed

ration was applied based on the biomass of Koi and Shing. All water quality parameters were deemed suitable for fish culture throughout the study period and differed only slightly between treatments (Table 7). Phytoplankton, zooplankton, and total plankton levels were all higher in T3, suggesting greater productivity within these ponds, although only the increase in zooplankton was significant (Table 8). Benthic organism abundance was also evaluated and determined to be highest overall in T1 (1562.69±8.08) and T2 (1369.00±10.5), followed by T3 (1198.35±51.9), and lowest in T4 (941.56±85.7) (Table 8). This could indicate that these organisms are being consumed by both the Shing and carps or that the presence of Shing within the ponds prevents these organisms from flourishing.

The highest gross production yield for Koi was observed in T2 (3634.17±9.22 kg/ha), followed closely by T3 (3594.27±9.33 kg/ha) and T4 (3547.92±11.72 kg/ha) with T1 being the lowest (3222.71±6.17 kg/ha). This is likely due to the higher survival rate in T2 as both T3 and T4 had higher specific growth rates (Table 9). For Rohu, the survival rate and gross production yield was greatest in T3 (93.5%, 1165.95±1.23 kg/ha) and lowest in T4 (82%, 1043.31±3.48 kg/ha). Similarly, Catla survival and production was greatest in T2 (92%, 452.79±0.42 kg/ha) and T3 (90%, 443.18±0.44 kg/ha) but lowest in T4 (79%, 389.47±0.32 kg/ha). Together, this suggests that the addition of Shing at low stocking densities is beneficial to the production of Koi and carps but higher densities of Shing are detrimental for these species. Gross production of Shing increased with increasing stocking density and was thus highest in T4 (Table 9), however the survival rates of Shing were 68%, 72%, and 67% in T2-4, respectively, again suggesting that lower stocking densities may be more beneficial overall. Further, the overall feed conversion ratio was significantly higher in T4 (1.86) relative to all other treatments (T1- 1.62, T2- 1.59, T3- 1.53) (Fig. 4).

Overall production was related to the stocking density of Shing within the ponds. Thus, the highest yield was observed in T4 (7130.56±14.43 kg/ha), followed by T3 (6766.99±9.17 kg/ha), T2 (5887.26±10.37 kg/ha), and T1 (4696.98±6.85 kg/ha) (Fig. 5). T4 also exhibited the highest gross return as Shing fetch a much higher market price (450 BDT) than the other three species (Koi- 180 BDT, Rohu- 170 BDT, Catla- 150 BDT) (Fig. 6). However, due to the increased cost of feed and the lower survival rates for all species at the highest stocking density of Shing, T3 produced the greatest net return at 676, 069±1946 BDT and highest Benefit Cost Ratio (BCR) of 1.72 (Table 10). T4 produced the second highest return and BCR (662,829±3505 BDT, 1.56), followed by T2 (402,250±1959 BDT, 1.49) and T1 (161,360±1224 BDT, 1.24). This result indicates that the addition of Shing to Koi-carp polyculture at moderate stocking densities is beneficial and could increase incomes and food production for rural fish farmers in Bangladesh.

CONCLUSION

The results of this investigation indicate that Koi and Shing are ideal candidates for polyculture with carps. The production of Koi is significantly higher in polyculture with Rohu and Catla than in monoculture, and the addition of moderate stocking densities of Shing ($2.0/m^2$) further enhanced growth and production of Koi as well as both carp species. Our data also shows that a 50% reduction in commercial feed application (feeding at 10-2.5% bw/day versus 20-5% bw/day) using the stocking densities employed in this study combined with weekly pond fertilization to support natural productivity can lower production costs without any negative impacts on fish growth or survival. Nutrient overloading often leads to poor water quality and disease outbreaks and thus reducing the amount of feed applied to the ponds could have positive environmental and socio-economic impacts. Although Koi and Shing fetch higher market value and have a higher nutrient content than carps, they are currently limited to monoculture and have high production costs from the use of commercial feeds which has prevented smaller farming households from culturing these species. Thus, adding Koi and Shing to existing carp culture systems and reducing feed application could allow an overall increase in production of both species in Bangladesh as well as enhance earnings and food availability for small-scale rural fish farmers.

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TABKES AND FIGURES

Table 3. Average water quality parameters from Study 1. Values are mean ± SEM. No significant differences were observed between treatments. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

	T1 (100% feed)	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Temperature (°C)	28.96±0.23	28.79±0.35	29.71±0.28	30.17±0.89
Transparency (cm)	36.58±2.53	32.75±3.05	36.92±3.68	31.25±2.97
Alkalinity (mg l^{-1})	85.50±7.05	79.33±6.24	78.67±6.22	84.75±6.75
pH	7.35±0.26	7.20±0.27	7.38±0.27	7.49±0.28
Dissolved Oxygen (mg l^{-1})	7.40±0.62	5.97±0.83	6.03±0.79	5.60±0.55
Nitrate (mg l^{-1})	0.01±0.007	0.01±0.004	0.03±0.013	0.01±0.004
Nitrite (mg l^{-1})	0.003±0.001	0.003±0.001	0.005±0.002	0.005±0.002
Ammonia (mg l^{-1})	0.07±0.02	0.15±0.05	0.17±0.04	0.23±0.07
Phosphate (mg l^{-1})	1.28±0.21	1.01±0.12	1.05±0.24	1.35±0.26
Chlorophyll-a (μg l^{-1})	200.1±40.9	230.4±49.1	182.1±39.6	189.5±25.7

Table 4. Plankton and benthic organism populations identified in Study 1. Values are mean abundance (×10^3 cells/L) ± SEM. No differences were observed between treatments. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

	T1 (100% feed)	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Plankton				
Phytoplankton	365.38±19.65	181.04±18.00	276.75±24.88	236.38±10.26
Zooplankton	25.08±2.77	33.96±1.44	44.67±4.15	58.25±5.31
Total Plankton	390.46±17.62	215.00±16.64	321.42±22.64	294.63±9.39
Benthic Organisms				
Oligochaeta	651.9±162.4	425.9±92.8	385.2±86.5	272.8±41.7
Chironomid Larvae	433.3±87.0	586.4±141.8	446.91±113.9	269.1±29.1
Mollusca	242.0±49.3	203.7±28.8	293.8±59.9	232.1±54.8
Unidentified	9.88±2.11	12.35±3.07	25.9±7.76	9.88±3.33
Total Benthos	1337.0±276.2	1228.4±249.1	1151.9±200.7	783.9±113.9

Table 5. Growth and production parameters for Study 1. Values are mean ± SEM. Values with different letters are significantly different (Tukey's HSD; $P < 0.05$). NA = not applicable. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

	T1 (100% feed)	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Koi (*A. testudineus*)				
Weight (g)	65.4 ± 0.20^{a}	97.3 ± 1.62^{ab}	120.4 ± 7.82^{b}	121.5 ± 1.32^{b}
Survival Rate (%)	60	47.5	55	57.35
Specific Growth Rate (% bw d^{-1})	3.79 ± 0.002^{a}	4.12 ± 0.014^{ab}	4.28 ± 0.058^{b}	4.31 ± 0.009^{b}
Gross Production (kg/ha)	1963.2 ± 5.87^{a}	2311.6 ± 38.4^{ab}	3311.0 ± 215^{bc}	3484.0 ± 37.8^{c}
Rohu (*L. rohita*)				
Weight (g)	NA	267.3 ± 12.7^{a}	297.1 ± 27.1^{a}	339.4 ± 29.7^{a}
Survival Rate (%)	NA	61.25	58.33	60
Specific Growth Rate (% bw d^{-1})	NA	2.08 ± 0.04^{a}	2.15 ± 0.07^{a}	2.26 ± 0.07^{a}
Gross Production (kg/ha)	NA	1309.5 ± 62.1^{a}	1386.4 ± 126.4^{a}	1629.1 ± 142.4^{a}
Catla (*C. catla*)				
Weight (g)	NA	492.8 ± 22.6^{a}	338.8 ± 29.1^{b}	396.3 ± 16.5^{ab}
Survival Rate (%)	NA	72.6	58	80
Specific Growth Rate (% bw d^{-1})	NA	2.26 ± 0.037^{a}	1.93 ± 0.073^{b}	2.08 ± 0.033^{ab}
Gross Production (kg/ha)	NA	715.5 ± 32.8^{a}	393.0 ± 33.8^{b}	634.1 ± 26.4^{a}
Total				
Feed Conversion Ratio	1.73 ± 0.005^{a}	1.53 ± 0.028^{ab}	1.31 ± 0.077^{bc}	1.03 ± 0.029^{c}
Gross Production (kg/ha)	1963.2 ± 5.87^{a}	4336.7 ± 77.9^{ab}	5090.4 ± 248^{bc}	5747.2 ± 164^{c}

Table 6. Economic analyses from Study 1. Values are mean ± SEM. Values with different letters are significantly different (ANOVA; $P < 0.05$). NA = not applicable. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

Financial Input *(BDT/ha)*	*T1 (100% feed)*	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Bleaching Powder	5928	5928	5928	5928
Lime ($CaCO_3$)	10,338	10,338	10,338	10,338
Urea	593	593	7824	7824
Triple Super Phosphate	445	445	5975	5975
Koi	81,814	81,814	81,814	81,814
Rohu	0	37,400	37,400	37,400
Catla	0	15,584	15,584	15,584
Feed	168,207	317,290	315,632	283,550
Labor	10,000	10,000	10,000	10,000
Total Cost (BDT/ha)	277,325	479,392	490,495	458,413
Total Production (kg/ha)	1963.2 ± 5.87^{a}	4336.7 ± 77.9^{ab}	5090.4 ± 248^{bc}	5747.2 ± 164^{c}
Gross Return (BDT/ha)	$353{,}376 \pm 1056^{a}$	$746{,}037 \pm 12670^{ab}$	$890{,}617 \pm 43361^{bc}$	$999{,}185 \pm 28185^{c}$
Net Return (BDT/ha)	$76{,}051 \pm 1056^{a}$	$266{,}\ 644 \pm 12670^{ab}$	$400{,}122 \pm 43361^{bc}$	$540{,}772 \pm 28185^{c}$
Benefit Cost Ratio (BCR)	1.27	1.56	1.82	2.18

Table 7. Water quality parameters from Study 2. Values are mean ± SEM. Different letters indicate significant differences (Tukey's HSD; $P < 0.05$).

	T1 (0/m² Shing)	*T2 (1/m² Shing)*	*T3 (2/m² Shing)*	*T4 (3/m² Shing)*
Temperature (°C)	28.96 ± 0.037^{a}	29.04 ± 0.037^{a}	29.26 ± 0.161^{a}	29.31 ± 0.046^{a}
Transparency (cm)	28.71 ± 0.66^{a}	28.47 ± 0.66^{a}	24.05 ± 1.18^{b}	27.43 ± 0.59^{ab}
Alkalinity (mg l^{-1})	78.63 ± 0.67^{a}	81.11 ± 1.00^{a}	90.07 ± 1.07^{b}	87.11 ± 1.22^{b}
pH	7.38 ± 0.01^{a}	7.43 ± 0.02^{a}	7.56 ± 0.02^{b}	7.53 ± 0.01^{b}
Dissolved Oxygen (mg l^{-1})	6.95 ± 0.05^{a}	7.10 ± 0.04^{ab}	7.31 ± 0.07^{b}	6.96 ± 0.04^{a}
Nitrate (mg l^{-1})	0.12 ± 0.002^{a}	0.11 ± 0.007^{a}	0.14 ± 0.007^{b}	0.09 ± 0.008^{a}
Nitrite (mg l^{-1})	0.13 ± 0.005^{a}	0.15 ± 0.006^{b}	0.11 ± 0.001^{a}	0.16 ± 0.005^{b}
Ammonia (mg l^{-1})	0.22 ± 0.013^{a}	0.21 ± 0.005^{a}	0.17 ± 0.003^{b}	0.21 ± 0.003^{a}
Phosphate (mg l^{-1})	0.87 ± 0.007^{a}	0.91 ± 0.013^{a}	0.81 ± 0.020^{a}	0.84 ± 0.009^{a}
Chlorophyll-a (µg l^{-1})	135.07 ± 0.36^{a}	146.40 ± 0.53^{b}	155.12 ± 0.78^{c}	154.60 ± 0.71^{c}

Table 8. Plankton and benthic organism populations identified in Study 2. Values are mean abundance ($\times 10^{3}$ cells/L) ± SEM. Different letters indicate significant differences (Tukey's HSD; $P < 0.05$).

	T1 (0/m² Shing)	*T2 (1/m² Shing)*	*T3 (2/m² Shing)*	*T4 (3/m² Shing)*
Plankton				
Phytoplankton	480.52 ± 38.9^{a}	467.22 ± 6.35^{a}	543.67 ± 18.6^{a}	462.78 ± 13.6^{a}
Zooplankton	15.96 ± 0.39^{a}	15.85 ± 1.24^{a}	24.85 ± 2.29^{b}	19.44 ± 0.84^{ab}
Total Plankton	496.48 ± 38.8^{a}	483.07 ± 5.14^{a}	568.52 ± 16.8^{a}	482.22 ± 13.2^{a}
Benthic Organisms				
Oligochaeta	618.38 ± 6.11^{a}	530.59 ± 9.23^{b}	469.68 ± 15.5^{c}	369.82 ± 21.1^{d}
Chironomid Larvae	225.93 ± 12.4^{a}	433.33 ± 15.8^{b}	446.91 ± 2.39^{c}	586.42 ± 6.33^{d}
Mollusca	392.87 ± 2.90^{a}	370.37 ± 13.3^{a}	348.97 ± 37.5^{a}	305.62 ± 60.3^{a}
Unidentified	20.30 ± 0.55^{a}	22.50 ± 4.88^{a}	18.11 ± 2.51^{a}	19.75 ± 3.43^{a}
Total Benthos	1562.69 ± 8.08^{a}	1369.00 ± 10.5^{ab}	1198.35 ± 51.9^{b}	941.56 ± 85.7^{c}

Table 9. Growth performance outcomes for Study 2. Values are mean ± SEM. Values with different letters are significantly different (Tukey's HSD; $P < 0.05$). NA = not applicable.

	T1 (0/m² Shing)	***T2 (1/m² Shing)***	***T3 (2/m² Shing)***	***T4 (3/m² Shing)***
Koi (*A. testudinius*)				
Harvesting Weight (g)	103.96 ± 0.20^{a}	103.83 ± 0.26^{a}	107.29 ± 0.27^{b}	109.17 ± 0.36^{c}
Harvesting Length (cm)	14.33 ± 0.16^{a}	14.04 ± 0.11^{a}	14.25 ± 0.14^{a}	14.54 ± 0.18^{a}
Survival Rate (%)	62	70	67	65
Specific Growth Rate (% bw d^{-1})	2.327 ± 0.001^{a}	2.326 ± 0.002^{a}	2.349 ± 0.002^{b}	2.376 ± 0.007^{b}
Gross Production (kg/ha)	3222.71 ± 6.17^{a}	3634.17 ± 9.22^{b}	3594.27 ± 9.33^{c}	3547.92 ± 11.72^{d}
Rohu (*L. rohita*)				
Harvesting Weight (g)	152.42 ± 0.15^{a}	153.17 ± 0.19^{ab}	155.88 ± 0.16^{bc}	159.04 ± 0.53^{c}
Harvesting Length (cm)	24.67 ± 0.19^{a}	25.73 ± 0.16^{b}	25.54 ± 0.16^{b}	25.58 ± 0.15^{b}
Survival Rate (%)	87	89	93.5	82
Specific Growth Rate (% bw d^{-1})	1.385 ± 0.003^{a}	1.386 ± 0.001^{ab}	1.396 ± 0.003^{bc}	1.410 ± 0.004^{c}
Gross production (kg/ha)	1060.82 ± 1.04^{ab}	1090.55 ± 1.34^{bc}	1165.95 ± 1.23^{c}	1043.31 ± 3.48^{a}
Catla (*C. catla*)				
Harvesting Weight (g)	243.21 ± 0.30^{a}	246.08 ± 0.23^{b}	246.21 ± 0.24^{b}	246.5 ± 0.20^{b}
Harvesting Length (cm)	27.91 ± 0.12^{a}	28.42 ± 0.14^{a}	28.17 ± 0.16^{a}	28.44 ± 0.19^{a}
Survival Rate (%)	85	92	90	79
Specific Growth Rate (% bw d^{-1})	1.366 ± 0.002^{a}	1.376 ± 0.002^{b}	1.373 ± 0.001^{b}	1.374 ± 0.001^{b}
Gross Production (kg/ha)	413.45 ± 0.32^{ac}	452.79 ± 0.42^{b}	443.18 ± 0.44^{bc}	389.47 ± 0.32^{a}
Shing (*H. fossilis*)				
Harvesting Weight (g)	NA	104.38 ± 0.13^{a}	108.58 ± 0.20^{b}	106.96 ± 0.30^{c}
Harvesting Length (cm)	NA	21.94 ± 0.04^{a}	22.01 ± 0.09^{a}	21.98 ± 0.12^{a}
Survival Rate (%)	NA	68	72	67
Specific Growth Rate (% bw d^{-1})	NA	2.535 ± 0.001^{a}	2.563 ± 0.001^{b}	2.553 ± 0.002^{c}
Gross Production (kg/ha)	NA	709.75 ± 0.85^{a}	1563.60 ± 2.92^{b}	2149.86 ± 5.99^{c}
Total				
Feed Conversion Ratio	1.62 ± 0.051^{a}	1.59 ± 0.055^{a}	1.53 ± 0.035^{a}	1.86 ± 0.049^{b}
Gross Production (kg/ha)	4696.98 ± 6.85^{a}	5887.26 ± 10.37^{b}	6766.99 ± 9.17^{c}	7130.56 ± 14.43^{d}

Table 10. Economic analysis for Study 2. Values are mean ± SEM. Values with different letters are significantly different (Tukey's HSD; $P< 0.05$).

Financial Input *(BDT/ha)*	***T1 (0/m² Shing)***	***T2 (1/m² Shing)***	***T3 (2/m² Shing)***	***T4 (3/m² Shing)***
Bleaching Powder	5928	5928	5928	5928
Lime ($CaCO_3$)	10,338	10,338	10,338	10,338
Urea	7231	7231	7231	7231
Triple Super Phosphate	5530	5530	5530	5530
Koi	49,400	49,400	49,400	49,400
Rohu	55,328	55,328	55, 328	55,328
Catla	21,736	21,736	21,736	21,736
Shing	0	49,400	98,800	148,200
Feed	495,594	609,708	674,916	865,326
Labour	10,000	10,000	10,000	10,000
Total Cost (BDT/ha)	661,085	824,599	939,207	1,179,017
Total Production (kg/ha)	4696.98±6.85[a]	5887.26±10.37[b]	6766.99±9.17[c]	7130.56±14.43[d]
Gross Return (BDT/ha)	822,445±1224[a]	1,226,849±1959[ab]	1,615,276±1946[bc]	1,841,847±3505[c]
Net Return (BDT/ha)	161,360±1224[a]	402,250±1959[ab]	676,069±1946[c]	662,829±3505[bc]
Benefit Cost Ratio	1.24	1.49	1.72	1.56

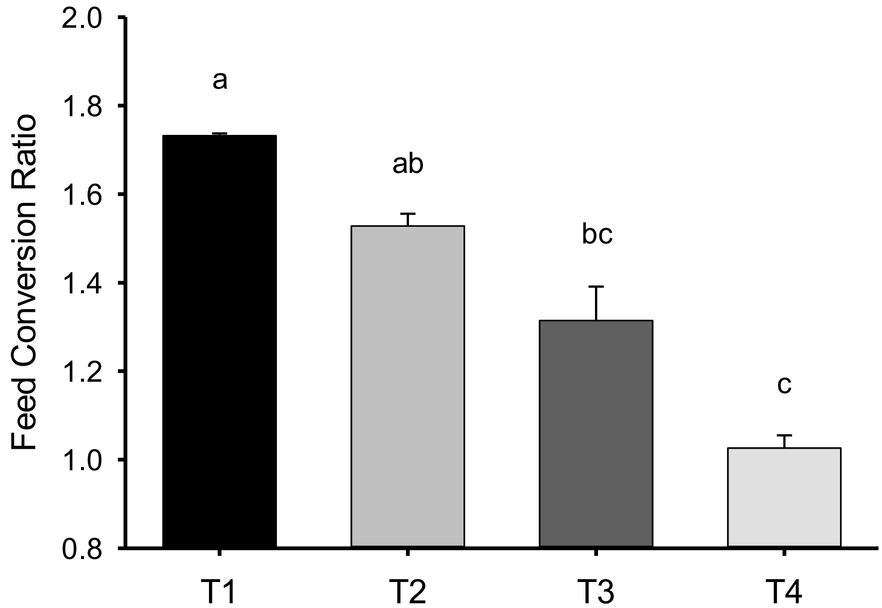

Figure 1. Feed conversion ratios for Koi monoculture (T1) and Koi-Carp polyculture (T2, 100% feed ration; T3, 75% feed ration; T4, 50% feed ration) in Study 1. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

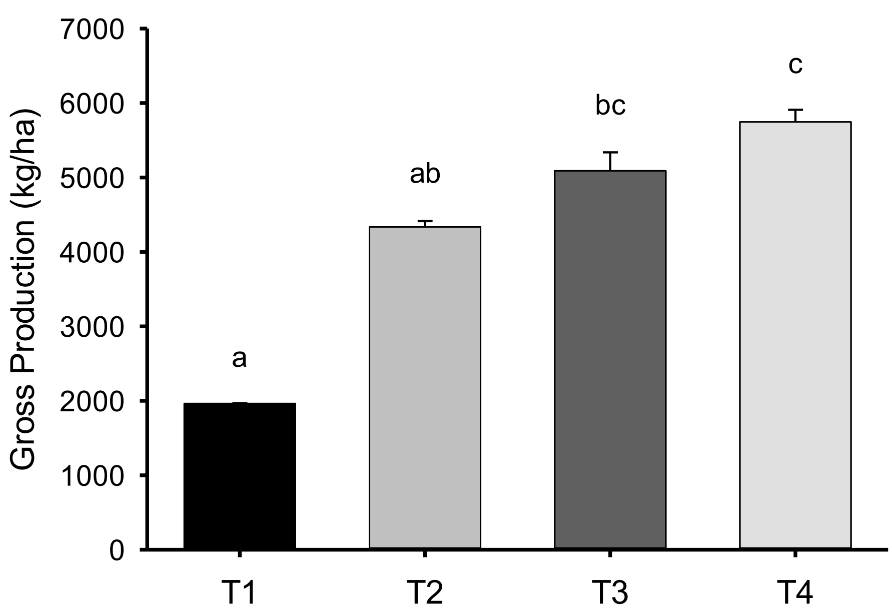

Figure 2. Gross production (kg/ha) for Koi monoculture (T1) and Koi-Carp polyculture (T2, 100% feed ration; T3, 75% feed ration; T4, 50% feed ration) in Study 1. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

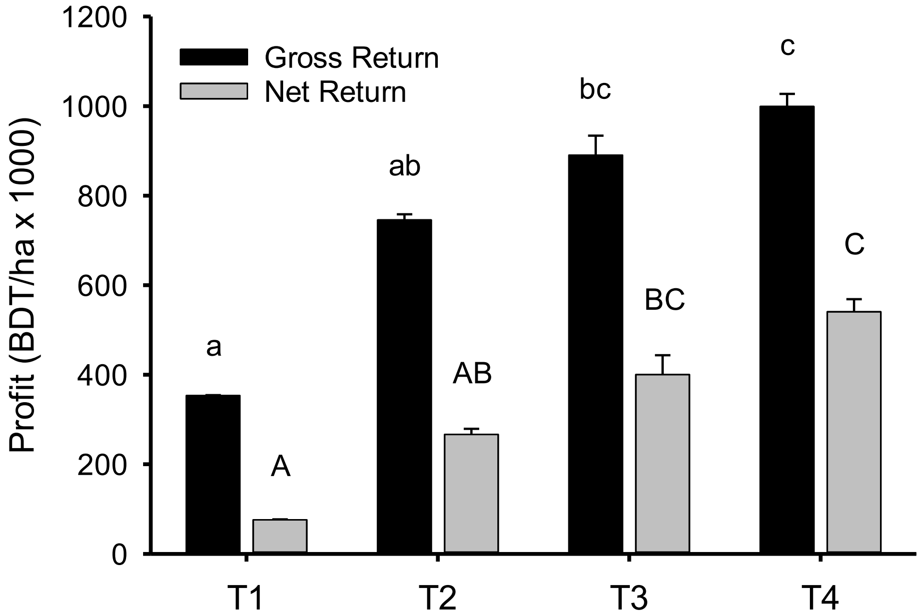

Figure 3. Gross (black) and net (grey) profits in Bangladesh Taka (BDT) for Koi monoculture (T1) and Koi-Carp polyculture (T2, 100% feed ration; T3, 75% feed ration; T4, 50% feed ration) in Study 1. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

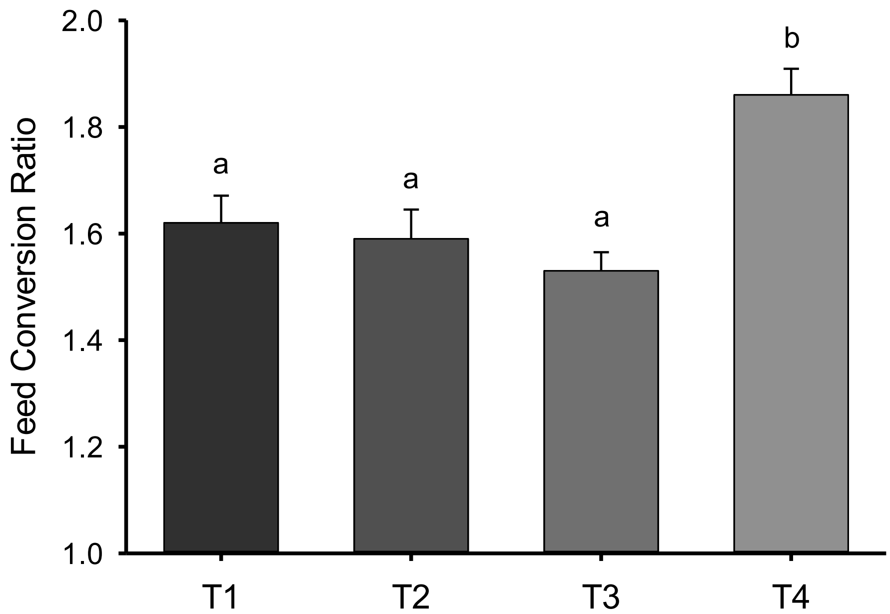

Figure 4. Feed conversion ratios for Koi-Carp (T1) and Koi-Carp-Shing (T2, 1/m^2 Shing; T3, 2/m^2 Shing; T4, 3/m^2 Shing) polyculture in Study 2. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

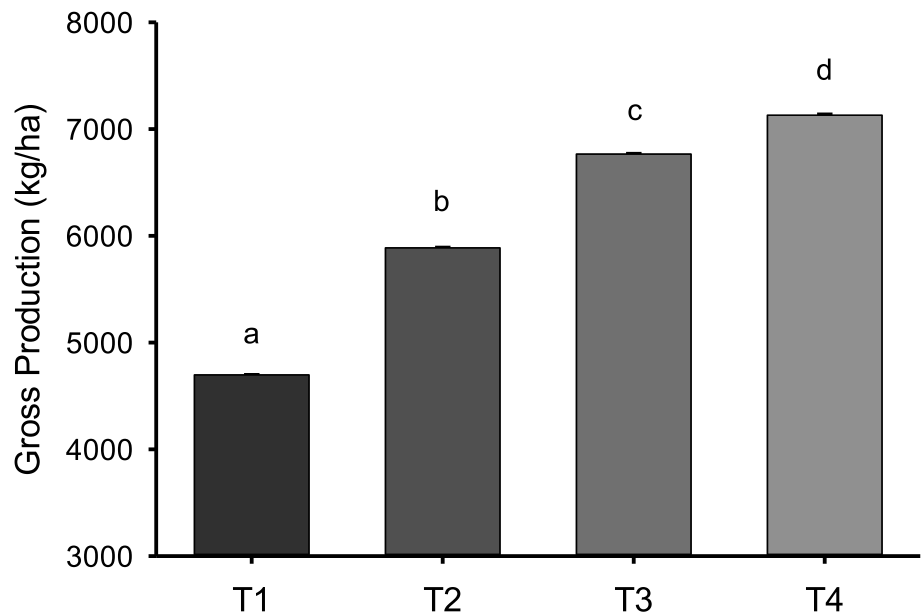

Figure 5. Gross production (kg/ha) for Koi-Carp (T1) and Koi-Carp-Shing (T2, 1/m^2 Shing; T3, 2/m^2 Shing; T4, 3/m^2 Shing) polyculture in Study 2. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; P < 0.05).

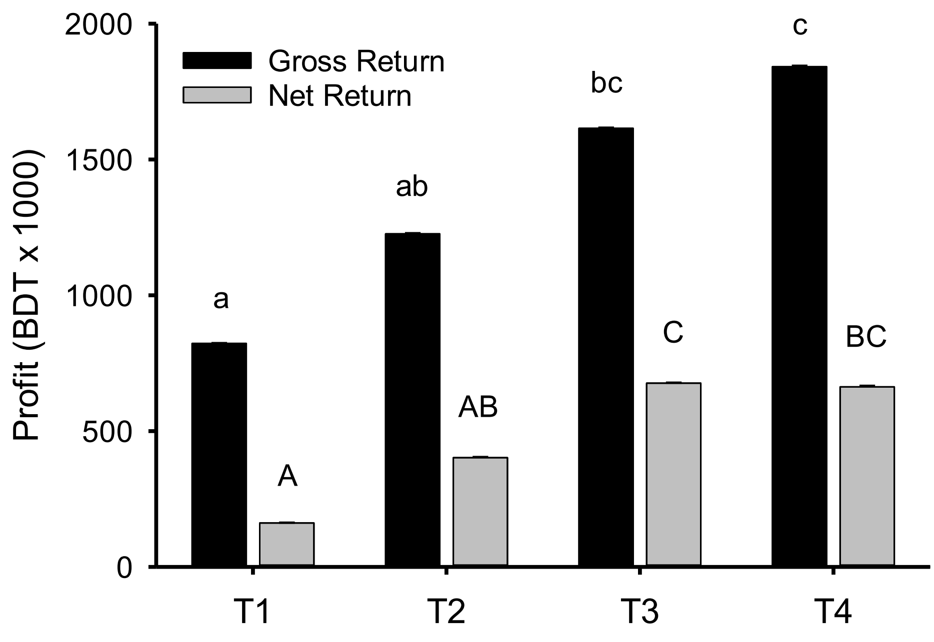

Figure 6. Gross (black) and net (grey) profits in Bangladesh Taka (BDT) for Koi-Carp (T1) and Koi-Carp-Shing (T2, 1/m^2 Shing; T3, 2/m^2 Shing; T4, 3/m^2 Shing) polyculture in Study 2. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; P < 0.05).

DISSEMINATION OF AQUAFISH INNOVATION LAB TECHNOLOGIES FOR IMPROVING FOOD PRODUCTION EFFICIENCY AND LIVELIHOODS OF THE PEOPLE OF BANGLADESH

Mitigating Negative Environmental Impacts/Activity/16MNE02NC

Shahroz Mahean Haque[1], Zahid Parvez Sukhan[2], Md. Lokman Ali[3], Khandaker Anisul Huq[4], Ashraful Islam[5], Kaniz Fatema[1], Sadika Haque[6], Russell Borski[7]

[1]Department of Fisheries Management, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh
[2]Department of Marine Fisheries and Oceanography, Patuakhali University of Science and Technology, Patuakhali, Bangladesh
[3]Faculty of Fisheries, Patuakhali University of Science and Technology, Patuakhali, Bangladesh
[4]Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh
[5]Department of Horticulture, BAU, Mymensingh, Bangladesh
[6]Department of Agricultural Economics, BAU, Mymensingh, Bangladesh
[7]Department of Biological Sciences, North Carolina State University, Raleigh, NC

ABSTRACT

The main goal of this extension and outreach activity is to promote improvements in management practices and new technologies developed by the AquaFish Innovation Lab that will allow farmers to enhance the efficiency and diversity of seafoods they produce while increasing their incomes and accessibility to nutrient rich foods. To this end, a series of 6 workshops and group training sessions was conducted to disseminate promising technologies. This included research on: 1) reduced feeding strategies for tilapia monoculture and polyculture with major Indian carps; 2) brackish water culture of *Pangasius* catfish and tilapia-*Pangasius* polyculture as a livelihood alternative for coastal farmers impacted by salinity incursion and contamination of freshwater farming systems; 3) use of locally-produced formulated diets to reduce costs and improve production efficiency of *Pangasius*; 4) integration of Mola, a small indigenous fish, and dyke cropping in traditional gher-pond freshwater prawn culture for production of nutrient rich fish and vegetables for home consumption; and 5) semi-intensive polyculture of Koi and Shing with Indian major carps as a new technology for sustainable production of high value, nutritious fishes. Presentations and extension leaflets outlining new and improved technologies for culturing fish and integrating vegetables in fish production were provided at the workshops. This along with industry stakeholder input, including from farmers who worked with the investigators on field trials, could help expand application of the new technologies to fisheries and aquaculture communities. The workshops were conducted in Mymensingh region of Northcentral, and in Barisal and Khulna regions of Southwest Bangladesh. Approximately 450 individuals participated in the workshops or training sessions. Farmers and industry stakeholders, including a large number of women constituted the majority of participants. Other participants came from 13 different NGOs, 7 universities, and 5 government institutes or agencies. A National Workshop focused on disseminating research impacts to government agencies and NGOs to better advance and promote upscaling of technologies and policies that improve resilience and outcomes of communities dependent on aquaculture and fisheries for their livelihood. A series of short-term training sessions also worked directly with women to include the importance and nutritional value of fish and of vegetables for family health and how contributions of Mola and vegetable dyke cropping can improve nutrient consumption and household livelihoods. Collectively, the extension and capacity building activities should go a long way toward expanding the new technologies to farming communities that can improve food security and nutrition of the Bangladesh people.

INTRODUCTION

Bangladesh is one of the most densely populated countries in the world with many living in abject poverty. Many of the women and children are malnourished with 38-55% exhibiting vitamin or mineral deficiencies and most relying on cereals (rice) for their nutrition. Aquaculture and fisheries make up a large proportion of employment opportunities for a majority of Bangladeshis in rural areas. Aquaculture in Bangladesh is considered a high food security priority for enhancing dietary nutrition and improving the economic livelihoods for its poorest citizens. Sustainable aquaculture is particularly important in the coastal plain regions of Southwest Bangladesh, where poverty is exceptionally high and the region is plagued by frequent flooding, saltwater incursion, and extreme weather (cyclones, seasonal drought, high temperature fluctuations) which are linked, in part, to global climate change. Aquaculture production in these regions and throughout Bangladesh face significant problems which directly threaten the lives and economic livelihoods of local farmers. These include: limited production of nutrient-rich foods available for direct consumption, poor productivity and high mortality rates, excessive and costly feed inputs leading to poor economic return, poor pond management leading to low water quality and environmental degradation, and limited diversification of aquaculture products. Our research is aimed at remediating some of these issues. Here we will disseminate the most promising results of our AquaFish Innovation Lab research in Bangladesh to farmers and their communities through a series of workshops and training sessions.

The main goal of this extension and outreach activity is to promote significant improvements in management practices and new technologies that will allow farmers to enhance the efficiency and diversity of seafoods they produce while increasing their incomes and accessibility to nutrient rich foods. These technologies incorporate practical methods for intensifying fish production in a sustainable manner while promoting production of fishes with high nutrient value. They also provide new ways to grow fish in environments impacted by global climate change, namely in water bodies afflicted by rising salinity. Among various projects, previous AquaFish Innovation Lab research shows, for instance, that incorporation of endemic, nutrient-dense small indigenous fish species (SIS) into gher-prawn farming coupled with pond dyke vegetable production provides farmers additional crops for home consumption as well as income from market sales, all while improving production of the prawn cash crop. Reducing daily ration of feed or feeding on alternate days can dramatically reduce costs and increase incomes for farmers without impacting overall yield of the cultivars produced. This research along with other studies indicate impacts of fish culture on environmental water quality and fish stock health can be improved through better management of feed inputs and by the incorporation of semi-intensive and polyculture production practices.

OBJECTIVES

1. Provide workshops and group training sessions to disseminate promising technologies derived from AquaFish Innovation Lab research.
2. Produce and distribute leaflets as outreach documents for extending aquaculture technologies to local farmers and the general public.
3. Work with local university, government, and NGO representatives to provide these outreach opportunities to the general public to enhance sustainability of project impacts.
4. Improve food production efficiency in an environmentally sustainable way to enhance nutrient consumption, incomes and the livelihoods of the people of Bangladesh.

MATERIALS AND METHODS

Location

Workshops were provided in 4 major regions of Bangladesh including where improved aquaculture management practices and new technologies were developed. The workshops were undertaken in the greater Mymensingh, Khulna, and Barisal regions of Bangladesh. A single, national workshop was held in Dhaka, the capital of Bangladesh. The workshops were cosponsored by local Bangladesh

Agricultural University, Khulna University, Patuakhali Science and Technology University and held at the university, village, or conference center in Dhaka, Bangladesh.

Methods

We worked with local university, government, and NGO representatives as well as extension agents to advertise the workshops and training sessions. These individuals where possible also attended, helped promote, and contributed to the outreach activities given to farmers and other stakeholders. This allowed for disseminating information in the best and most accepted methods in the relevant areas. We used a combination of extension leaflets or brochures developed by the PIs as well as presentations outlining the improved management practices and aquaculture technologies promising aquaculture technologies. Materials were also distributed to local extension agencies and NGOs. Where applicable, pond-side training was also conducted.

Five 1-day workshops and a series of training sessions were provided in each of the four regions of Bangladesh: Khulna, Patuakhali, Mymensingh, and Dhaka (a total of 6 workshops). Presentations focused on the aquaculture developments that came from AquaFish research in each of the regions (see below). At least 25 individuals, and in most case far more attended the workshops. We encouraged women in the farming community to attend to learn about better aquaculture practices, as they are often responsible for taking care of food crops and provide nutrition to their children. We invited farmers with whom we conducted on-farm trials to test new technologies and their benefits. Farmer-to-farmer extension of new culture technologies can increase the likelihood that farming households incorporate the new practices into their operations. Our participant farmers could also serve as liaisons to other farmers in the area should they want to adopt new practices.

Provisions for transport to workshops was provided to those who required it. Lunch, snacks, tea, and soft drinks were provided for the day's events.

The title, date(s) and location of the workshops are as follows:

***Workshop 1*:**
Title: Training Workshop on Culture of Pangas, Tilapia, and Koi in Hypo-saline Water
Date: November 28, 2017
Location: Hazipur (Village), Kalapara (Upazila), Patuakhali (District), Barisal (Division), Bangladesh

***Workshop 2*:**
Title: Training Workshop on Culture of Pangas, Tilapia, and Koi in Hypo-saline Water
Date: December 01, 2017
Location: Anipara, Kalapara, Patuakhali, Bangladesh

***Workshop 3 (Four training sessions, 25 persons/session)*:**
Title: Integration of Nutrient-rich fish SIS and vegetables with Prawn-Carp Gher farming in Southwest Bangladesh
Date: December 15, 2017; December 26, 2017; January 5, 2018; January 15, 2018
Location: Khulna University, Khulna, Bangladesh

***Workshop 4*:**
Title: Integration of Nutrient-rich fish SIS and vegetables with Prawn-Carp Gher farming in Southwest Bangladesh
Date: December 21, 2017
Location: Khulna University, Khulna, Bangladesh

***Workshop 5*:**
Title: Polyculture of Air breathing Fishes, Koi, and Shing with Indian Major Carps for Enhancing Income and Dietary Nutrition while Reducing Environmental Impacts
Date: May 31, 2018
Location: Bangladesh Agricultural University, Mymensingh, Bangladesh

***Workshop 6*:**
Title: Dissemination of AquaFish Innovation Lab Findings
Date: January 15, 2018
Location: Krisibid Institute, Farmgate, Dhaka, Bangladesh

RESULTS AND DISCUSSION

Workshops 1 and 2

There were 47 farmers who participated at Workshop 1 (17 women, 30 men) and another 50 farmers at Workshop 2 (20 women, 30 men). Additionally, 4 local representatives and 4 faculty and 5 students from Patuakhali University of Science and Technology attended each of the workshops providing a total of 118 participants at both workshops (Figure 1). The workshops disseminated on-farm research that demonstrate *Pangasius* catfish (*Pangasius hypopthalamus*) can be grown in brackish waters as effectively as in freshwater ponds. This provides a new livelihood alternative for farmers impacted by rises in surface water salinities seen in coastal regions of Bangladesh. Incorporating less costly, locally made formulated feeds also provides additional cost benefits and improves returns on investment for culturing *Pangasius*. This was then followed up by discussions on research showing that Nile tilapia (*Oreochromis niloticus*) is the more optimal candidate for polyculture with *Pangasius* in hyposaline waters with *Pangasius*-Koi (the freshwater climbing perch, *Anabas testudineus*) polyculture providing the second greatest profit margin relative to *Pangasius* culture alone. Polyculture of all three species together is less viable, likely from competition between Koi and tilapia. Participatory discussion sessions that included input from farmers who were involved in the demonstration research studies were also conducted and farmers had the opportunity to visit the field sites where research took place to demonstrate the methods and benefits of the new culture technologies.

Workshop 3

From October 2017 to January 2018, 100 farmers were trained on technologies for integrating Mola, a small indigenous fish with high nutritional value, and dyke cropping for vegetable production into prawn-carp-gher farming systems. Incorporating Mola, carps, or both into current prawn-gher farming practices does not adversely impact, but in fact enhances the production of prawn. In utilizing these practices, farming families can obtain additional nutrients from domestic consumption of Mola while improving production of prawn as a cash crop. AquaFish Innovation Lab research also established that pond muds derived from prawn-fish polyculture in ghers are more effective than soil in producing vegetables on pond dykes, providing an additional source of nutritional food for consumption or sale. The best types of fertilizers to use in growing vegetables (winter and summer types including fruits) and fish in the integrated aquaculture-agriculture system was disseminated.

The training was conducted in four slots with 25 farmers per slot and more than 63% of the participants were female (63 female, 37 male). Women are primarily responsible for nutrition in farming households and also contribute to gher farming by feeding fish and cultivating dyke vegetables. Training was provided to the participants to include the importance and nutritional value of fish (with emphasis on SIS) and of vegetables to family health and how contributions of Mola and vegetable dyke cropping can improve nutrient consumption and household livelihoods. Participants were provided training on nursery and grow out pond preparation, dyke cropping, nursing of prawn post-larvae, liming importance and dosage, fertilization dosage and application, fish stocking, feed, and feeding, pond productivity, sampling and regular health checks, common disease/parasites, safe harvesting, and proper washing, transport, and marketing. Farmers received training on record book keeping as well. Leaflets outlining the new integrative agriculture-aquaculture technologies developed from AquaFish Innovation Lab studies were distributed among participants. Local fish farmers have been or are inspired to incorporate Mola fish and dyke vegetables into their gher culture production systems through discussions and observations of farmers who undertook initial testing trials. Many have initiated the new culture technologies for integrating Mola and dyke vegetables into their traditional farming systems.

Workshop 4
The AquaFish Innovation Lab research team from Khulna University organized an interactive workshop on December 21, 2017 entitled "*Integration of Nutrient-rich small fish and vegetables with Prawn-Carp Gher farming in Southwest Bangladesh*" to disseminate the outcomes of the new technologies developed (Figure 2). More than 50 attendees from various organizations and the local farming community attended the workshops. They included representatives from the Department of Fisheries (DoF), Bangladesh Fisheries Research Institute (BFRI), Department of Agriculture Extension (DAE), Soil Resource Development Institute (SRDI), various development NGOs (WorldFish, SOLIDARIDAD, Blue Gold, Winrock International, Shushilan, Rupantor, Prodipon, Nobolok, Ashroy Foundation), faculty from various universitites, and local gher farmers. Leaflets on the research findings were distributed to the participants. As the Department of Fisheries is the government organization responsible for disseminating fisheries-related technologies to local farmers, the leaflets were also distributed to officers at various levels within the department, e.g. Deputy Director, District Fisheries Officer, Upazilla Fisheries Officers of Khulna Division. We believe that through these sectors thousands of farmers will be informed of the new technologies. Information from the workshop was also highlighted in six different Bangladeshi newpapers.

Workshop 5
This workshop disseminated research showing: 1) Koi and Shing (*Heteropneustes fossilis*), air breathing fishes high in commercial and nutrient value, can be grown more profitably in polyculture with major Indian Carps (Rohu and Catla) than in monoculture in semi-intensive pond production systems, 2) reducing daily feed ration by 50% from that typically used in intensive monoculture provides additional cost benefits to the polyculture of these fishes, and 3) polyculture of Koi-carp with Shing may be even more profitable than Koi-carp polyculture. The workshop had 82 participants that included 70 fish farmers and hatchery operators in the Mymensingh region of central Bangladesh as well as fisheries managers, students, journalists, and faculty from Bangladesh Agricultural University (BAU) (Figure 3). The workshop was held for a full day at BAU where the new technologies and their application were presented to farmers. Extension leaflets were provided and farmers had the opportunity to discuss their experiences and successes as well as methodology for applying the new technologies to their aquaculture operations. Field visits to the research farm were also provided to all participants. The workshop received press coverage through http://shobujbangladesh24.com (http://shobujbangladesh24.com/?p=27889).

Workshop 6
This National workshop was undertaken in Dhaka, the capital of Bangladesh, and focused on disseminating all aquaculture and fisheries technologies developed by the AquaFish Innovation Lab project to primarily government agencies (Department of Fisheries, Bangladesh Fisheries Research Institute, Department of Agricultural Extension) as well as NGOs (WorldFish, Helen Keller International, Sushilan, BRAC Centre, Hortex Foundation, CARE) (Figure 4). This is critical to the overall exit strategy, aimed at maintaining support for programs begun by the AquaFish Innovation Lab, and to advance and promote upscaling of technologies in the aquaculture and fisheries sectors developed by the AquaFish Innovation Lab. There were 67 participants at the workshop from various

government and nongovernment agencies, universities, and private industry. The Director General of the Bangladesh Department of Fisheries and colleagues attended the workshop. The Director presented a synopsis on the state of fisheries and aquaculture in Bangladesh and the importance of AquaFish Innovation Lab research in promoting priority areas of growth in the sector needed for food security. Highlights on novel and improved fish and shellfish culture technologies for enhancing food production efficiency and sustainability while increasing household incomes and fish consumption were covered at the workshop by AquaFish Innovation Lab researchers and their partners.

The technologies developed and disseminated at the workshop include:
1. Reduced feeding strategies for tilapia monoculture and polyculture with major Indian carps.
2. Brackish water culture of *Pangasius* catfish and tilapia-*Pangasius* polyculture as a livelihood alternative for coastal farmers impacted by salinity contamination of freshwater farming systems.
3. Use of locally produced formulated diets to reduce costs and improve production efficiency of *Pangasius*.
4. Addition of Mola and dyke cropping to traditional gher-pond freshwater prawn culture for production of nutrient rich fish and vegetables for home consumption.
5. Semi-intensive polyculture of Koi and Shing with Indian major carps as a new technology for sustainable production of the high value, nutritious fishes.

Several farmers who undertook aquaculture studies with AquaFish Innovation Lab researchers presented their experiences and successes in producing and marketing aquafoods. Overall, this workshop was very successful in that it conveyed the impact of research done by the AquaFish Innovation Lab and provided a discussion of future needs and opportunities for promoting food production and nutrition in Bangladesh.

CONCLUSION

Six workshops or short-term training sessions were undertaken to disseminate AquaFish Innovation Lab technologies for improving food production efficiency and livelihoods of the people of Bangladesh. The workshops were carried out in three regions of Bangladesh where research established novel and improved methods for fish production. The regions were Mymensingh of Northcentral Bangladesh and Khulna and Barisal in Southwest Bangladesh, the latter reflecting the high priority zone of influence for the USAID Feed the Future program on Global Hunger and Food Security. Approximately 450 individuals participated in the workshops or training sessions. Farmers and industry stakeholders, including a large number of women constituted the majority of participants. Other participants came from 13 different NGOs, 7 universities, and 5 government institutes or agencies. Faculty, students, farmers, and others provided presentations on the most promising new aquaculture management practices and technologies that were shown to improve fish yields, farmer incomes, and consumption of nutritious seafoods. Some also promote resilience, including mitigating impacts of seawater incursion along coastal lands by incorporating fish that can be grown in brackish water environments. Several extension leaflets produced by AquaFish Innovation Lab researchers that outline new and improved management practices were distributed to participants, including NGOs and government agencies whose goals are to promote and facilitate upscaling of technologies. Collectively the activities conducted herein, including that derived from a National Workshop, should go a long way toward extending aquaculture technologies to Bangladesh communities that were tested and shown to improve food security and nutrition in Bangladesh.

FIGURES

Figure 1. Pictures from workshop 1 and 2 held in the Barisal region of Bangladesh. Includes a participant farmer speaking of his experience with pond trials conducted with AquaFish Innovation Lab researchers.

Figure 2. Chairwoman delivering a presentation at Workshop 4 held at Khulna University (left panel) and workshop participants representative of the farming community, aquaculture industry, NGOs, government fisheries agencies, university research and extension programs, and local media organizations.

Figure 3. Pictures of Workshop 5 participants at the conference venue (left panel) and at research field sites (right panel) at Bangladesh Agricultural University.

Figure 4. Some Participants at the National Workshop on Dissemination of AquaFish Innovation Lab Research (left panel). The right panel shows some of the faculty and students that makeup the AquaFish Innovation Lab Research Team in Bangladesh.

APPENDIX I. INVESTIGATIONS SUMMARY & ASSESSMENT

Appendix 1. AquaFish Innovation Lab 2016-2018 Investigation Summary and Assessment.

Investigation Code	Investigation Title	Lead US Project PI	Lead US Institution	Final Status*
16BMA01PU	Experimental Pond Unit Assessment in Tanzania	Kwamena Quagrainie	Purdue University	1
16BMA02PU	Optimizing the Use of Commercial Feeds in Semi-Intensive Pond Production of Tilapia in Ghana; From Nursery to Grow-Out	Kwamena Quagrainie	Purdue University	1
16BMA03UM	A Comparison of Monoculture and Polyculture of Tilapia with Carps for Pond Production Systems in Nepal	James Diana	University of Michigan	1
16BMA04UM	Developing New Systems for Periphyton Enhancement in Farmers' Ponds	James Diana	University of Michigan	1
16BMA05AU	Water, Water Quality, and Pond Bottom Soil Management in Ugandan Aquaculture	Joseph Molnar	Auburn University	1
13BMA01PU	Coastal Women's Shellfish Aquaculture Development Workshop	Kwamena Quagrainie	Purdue University	1
13BMA05AU	Development of Low-Cost Aquaponics Systems for Kenya	Joseph Molnar	Auburn University	1
16SFT01UC	Pellet Feed Improvements Through Vitamin C Supplementation for Snakehead Culture	Robert Pomeroy	University of Connecticut	1
16SFT02NC	Nutritional Conditioning During Larval Development to Improve Feed Efficiency and Identify Beneficial Gut Flora in Tilapia	Russell Borski	North Carolina State University	1
16SFT03PU	Increasing Productivity of Nile Tilapia (*Oreochromis niloticus*) Through Enhanced Feeds and Feeding Practices	Kwamena Quagrainie	Purdue University	1
13SFT02PU	Enhancing the Nutritional Value of Tilapia for Human Health	Kwamena Quagrainie	Purdue University	1
13SFT06AU	Assessment of Growth Performance of Monosex Nile Tilapia (*Oreochromis niloticus*) in Cages Using Low-Cost, Locally Produced Supplemental Feeds and Training Fish Farmers on Best Management Practices in Kenya	Joseph Molnar	Auburn University	1
16IND01UC	Sustainable Snakehead Aquaculture in Cambodia	Robert Pomeroy	University of Connecticut	1
16IND02NC	Tilapia and Koi (Climbing Perch) Polyculture with *Pangasius* Catfish in Brackish (Hyposaline) Waters of Southern Bangladesh	Russell Borski	North Carolina State University	1
16IND03AU	Development of Captive Breeding, Larval Rearing Technologies and Management Practices for African Lungfish (*Protopterus aethiopicus*)	Joseph Molnar	Auburn University	1
16IND04MS	Develop a Conceptual Model to Evaluate the Potential Changes in Inland Food Fish Supply Under Various Global Climate Change Scenarios	William Taylor	Michigan State University	1

Investigation Code	Investigation Title	Lead US Project PI	Lead US Institution	Final Status*
16QSD01UC	Genetic diversity of striped snakehead (*Channa striata*) in Cambodia and Vietnam	Robert Pomeroy	University of Connecticut	1
16QSD02UM	Improving Seed Production of Sahar (*Tor putitora*) in Chitwan Nepal	James Diana	University of Michigan	1
13QSD01PU	Spat Collection and Nursery Methods for Shellfish Culture by Women	Kwamena Quagrainie	Purdue University	1
16HHI01NC	Better Management Practices for Mola-Prawn-Carp Gher Farming Integrated with Pond Dyke Cropping for Increased Household Nutrition and Earnings of Rural Farmers in Southwest Bangladesh	Russell Borski	North Carolina State University	1
16HHI02PU	Fish Consumption and Implications for Household Nutrition and Food Security in Tanzania and Ghana	Kwamena Quagrainie	Purdue University	1
16HHI03UM	Outreach to Increase Efficiency of Aquaculture in Nepal	James Diana	University of Michigan	1
16HHI04AU	Women in Uganda Aquaculture: Nutrition, Training, and Advancement	Joseph Molnar	Auburn University	1
16HHI05MS	Determine the Role of Wild-Caught and Aquaculture-Based Inland Fisheries in Meeting Burma's Human Nutritional Needs	William Taylor	Michigan State University	1
16FSV01UC	Enhancing Food Safety and Household Nutrition of Women and Children through Aquaculture and Capture Fisheries in Cambodia and Vietnam in the Dry Season	Robert Pomeroy	University of Connecticut	1
16FSV02AU	Implementing and Assessing Cell-Based Technical and Marketing Support Systems for Small and Medium-scale Fish Farmers in Uganda	Joseph Molnar	Auburn University	1
16PDV01UC	Guidance and policy recommendations for sustainable snakehead aquaculture and aquatic resource management in Cambodia and Vietnam	Robert Pomeroy	University of Connecticut	1
16MER01PU	Enhancing the Functionality and Applicability of Fish Market Information System (FMIS) to Marine Artisanal Fisheries in Ghana	Kwamena Quagrainie	Purdue University	1
16MER02AU	Assessment of Price Volatility in the Fish Supply Chain in Uganda	Joseph Molnar	Auburn University	1
16MNE01NC	Advancing Semi-Intensive Polyculture of Indigenous Air-Breathing Fishes, Koi and Shing, with Major Indian Carps for Enhancing Incomes and Dietary Nutrition while Reducing Environmental Impacts	Russell Borski	North Carolina State University	1
16MNE02NC	Dissemination of AquaFish Innovation Lab Technologies for Improving Food Production Efficiency and Livelihoods of the People of Bangladesh	Russell Borski	North Carolina State University	1

*Final Status of FIRs was determined based on the following criterias:

1- FIR fulfilled all AquaFish IL Management Team requirements (A. demonstrated that Work Plan objectives were met, B. complied with the report stucture instructions, and C. represented high quality work) by 15 June 2018, the deadline for inclusion in this Technical Report.
2- FIR was submitted, but did not meet all AquaFish IL Management Team requirements by 15 June 2018, and therefore was not included in this Technical Report.
3- FIR was not received by the AquaFish IL Management Team by 15 June 2018, and therefore was not included in this Technical Report.