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

IMPLEMENTATION PLAN 2016-2018

Published December 2016

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

USAID FROM THE AMERICAN PEOPLE · Oregon State University OSU · AQUAFISH INNOVATION LAB

AQUAFISH INNOVATION LAB
IMPLEMENTATION PLAN 2016-2018

Program activities are funded in part by the United States Agency for International Development (USAID) under CA/LWA No. EPP-A-00-06-00012-00 and by participating US and Host Country institutions.

The mission of the AquaFish Innovation Lab is to enrich livelihoods and promote health by cultivating international multidisciplinary partnerships that advance science, research, education, and outreach in aquatic resources. Bringing together resources from Host Country institutions and US universities, the AquaFish Innovation Lab emphasizes sustainable solutions in aquaculture and fisheries for improving health, building wealth, conserving natural environments for future generations, and strengthening poorer countries' ability to self-govern.

Cover Photo
Earthen ponds used for culturing fish, Bangladesh. 2014 Photo Courtesy of Russell Borski.

Acknowledgements
The AquaFish Management Entity acknowledges the contributions of researchers and the support provided by participating US and Host Country institutions, including the associated collaborators involved in this work. The editors acknowledge the contributions of Ford Evans, Kat Goetting, Stephanie Ichien, and Briana Goodwin.

This publication may be cited as:
AquaFish Innovation Lab. December 2016. Borberg, J., Hyman, A. A., and Egna H. (eds). Implementation Plan 2016-2018. AquaFish Innovation Lab, Oregon State University. Corvallis, Oregon, USA.

AquaFish Innovation Lab
College of Agricultural Sciences
Oregon State University
Strand Agriculture Hall
Corvallis, Oregon 97330-1643 USA

INTRODUCTION

The Feed the Future Innovation Lab for Collaborative Research on Aquaculture & Fisheries (AquaFish), previously the AquaFish Collaborative Research Support Program, is operating under a five-year extension from USAID that was awarded in 2013. USAID (May 2013, RFA) looks to AquaFish to "develop more comprehensive, sustainable, ecological and socially compatible, and economically viable aquaculture systems and innovative fisheries management systems in developing countries that contribute to poverty alleviation and food security." As part of the USAID Feed the Future Food Security Innovation Center, AquaFish operates under the Program for Research on Nutritious and Safe Foods, which "addresses undernutrition, especially in women and children, by increasing the availability and access to nutrient dense foods through research on horticulture crops, livestock, fish and dairy, food safety threats such as mycotoxins and other contaminants and on household nutrition and food utilization" (R. Bertram, 7 December 2012).

AquaFish is managed to achieve maximum program impacts, particularly for small-scale farmers and fishers, in Host Countries and more broadly. AquaFish's program objectives address the need for world-class research, capacity building, and information dissemination. Specifically, AquaFish strives 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

The overall research context for the projects described in this Implementation Plan is poverty alleviation and food security improvement through sustainable aquaculture development and aquatic resources management. Research that builds on existing work to generate new information and establishes an exit strategy so that research and impacts can extend beyond the life of the AquaFish Innovation Lab forms the core of AquaFish 2016-2018 projects. Projects also integrate institutional strengthening, gender, outreach, and capacity building through acti
vities 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 are now being built upon 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.

The five research projects described herein represent all of the key regions, themes, and topic areas called for in the AquaFish RFP issued in 2015 by Oregon State University (OSU), which serves as the overall Lead Institution for AquaFish. The five projects involve multiple lines of research and capacity building, accounting for over US $3 million from USAID, not including management costs or other outreach expenditures. In addition, each project targets 25% in matching funding for each federal dollar received.

PROGRAM REGIONS

Projects were selected that focus on established AquaFish Host Countries in Asia and Africa. As a group, these projects include 8 Host Countries, 12 US universities, and 26 Host Country (HC) institutions in formal funded partnerships. The eight Host Countries include Ghana, Tanzania, Kenya, Uganda, Bangladesh, Nepal, Vietnam, and Cambodia (all projects focus on at least one USAID-FTF Focus Country). Proposed activities received USAID country-level concurrence prior to award. Attention was paid to projects aimed at strengthening existing countries' infrastructure to build on previous USAID investments. Project proposals were evaluated based on the strength of linkages to host countries, among other criteria. Projects extend supportive activities to nearby countries within the same region. Existing AquaFish relationships are utilized throughout the research projects.

GLOBAL THEMES (GOALS)

The four global themes of AquaFish are cross-cutting and address several specific USAID policy documents and guidelines, including the Feed the Future Initiative and Research Strategy. Each project focuses on one primary AquaFish theme, yet balances 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 are organized around ten specific areas of inquiry called Topic Areas that pertain to aquaculture and the nexus between aquaculture and fisheries. AquaFish takes a systems approach that incorporates 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 and Best Management Alternatives (BMA)
Aquaculture is an agricultural activity with specific input demands. Systems need to be designed to improve efficiency and/or integrate aquaculture inputs and outputs with other agricultural and non-agricultural production systems. AquaFish research must benefit smallholder or low- to semi-intensive producers, and should focus on low-trophic species for aquaculture development. Design systems to limit negative environmental impacts, to improve overall fish health, and optimize carrying capacity. Interventions for disease and predation prevention must adopt an integrated pest management (IPM) approach and be careful to consider consumer acceptance and environmental risk of selected treatments. Innovative research is encouraged on: recirculating and aquaponics systems for supplying aquatic products to denser marketplaces in urban and peri-urban areas; integrated systems using shellfish, seaweeds, or other plants and animals; and new solutions for aeration, cold storage, and pond operations involving solar or other novel energy sources.

Sustainable Feed Technology and Nutrient Input Systems (SFT)
Methods of increasing the range of available ingredients and improving the technology available to manufacture and deliver feeds are a critical research theme. Better information about fish nutrition can lead to the development of less expensive and more efficient feeds. Investigations on successful adoption, extension, and best practices for efficient feed strategies that reduce the "ecological footprint" of a species under cultivation are encouraged. Research on soil-water dynamics and natural productivity to lessen feed needs were fundamental to the PD/A and ACRSPs; critical new areas of research may be continued, along with outreach to poor farmers using low- cost, no/low-feed technologies. Feed research that lessens reliance on fishmeal/proteins/oils and lowers feed conversion ratios is desired, as is research on feeds (ingredients, sources, regimes, formulations) that result in high quality and safe aquaculture products with healthy nutrition profiles. Complex pond dynamics technologies need to be simplified for use by new farmers; improved applications of pond dynamics technologies for driving non-fed plankton-driven systems is applicable where access to feeds is expensive or unreliable.

Climate Change Adaptation: Indigenous Species Development (IND)
Aquaculture, like agriculture and other human activities, will feel the effects of long-term climate change. Among the myriad challenges, ocean acidification and sea level rise will affect the world's coastal aquaculture operations, much of which occurs in poorer countries. Temperature changes will test the resiliency of domesticated varietals. Research challenges involve understanding the adaptive range of these species, and developing cultivation techniques for new species, such as air-breathing fishes. The shifting distribution of global freshwater supplies will pose challenges for the aquaculture industry, small farmers, and the marketplace. Genomics tools may be used to characterize candidate air-breathing species already being evaluated through previous CRSP research. Domestication of indigenous species may contribute positively to the development of local communities as well as protect ecosystems. At the same time, the development of new native species for aquaculture must be approached in a responsible manner that diminishes the chance for negative environmental, economic, and social impacts. Research that investigates relevant policies and practices is encouraged while exotic species development and transfer of non-native fishes are not encouraged. A focus on biodiversity conservation and biodiversity hotspots, as related to the development of native species for aquaculture, is of great interest. Aquaculture, done sensitively, can be a means to enhance and restock small-scale capture and wild fisheries resources. (Aquaculture-Fisheries Nexus Topic Area)

Quality Seedstock Development (QSD)
Procuring reliable supplies of high quality seed for stocking local and remote sites is critical to continued development of the industry, and especially of smallholder private farms. A better understanding of the factors that contribute to stable seedstock quality, availability, and quantity for aquaculture enterprises is essential. Genetic improvement (e.g., selective breeding) that does not involve genetically modified organisms (GMOs) may be needed for certain species that are internationally traded. All genetic improvement strategies need to be cognizant of marketplace pressures and trends, including consumer acceptance and environmental impacts. Augmentation of bait fisheries through aquaculture to support capture fisheries is an area of interest, provided there are no net negative environmental effects.

People, Livelihoods, and Ecosystem Interrelationships
Human Nutrition and Human Health Impacts of Aquaculture (HHI)
Aquaculture can be a crucial source of protein and micronutrients for improved human health, growth, and development. Research on the intrinsic food quality of various farmed fish for human consumption is needed—this might include science-based studies of positive and negative effects of consuming certain farmed fishes. Patterns of fish consumption are not well understood for many subpopulations. Human health can be negatively impacted by aquaculture if it serves as a direct or

indirect vector for human diseases. There is interest in better understanding the interconnectedness of aquaculture production and water/vector- borne illnesses such as malaria, schistosomiasis, and Buruli ulcer and human health crises such as HIV/AIDS and avian flu. Focus on vulnerable populations, women and children, and underserved populations, and assess how any given technology will affect or improve the welfare of these groups. Research or field-testing with schools and nutrition centers is encouraged. **(**Aquaculture-Fisheries Nexus Topic Area)

Food Safety, Post Harvest, and Value-Added Product Development (FSV)
Ensuring high quality, safe, and nutritious fish products for local consumers and the competitive international marketplace is a primary research goal. Efforts that focus on reducing microbial contamination, HACCP (Hazard Analysis and Critical Control Point) controls and hazards associated with seafood processing, value-added processing, post-processing, and by-product/waste development are of interest. Consumers and producers alike will benefit from research that contributes to the development of standards and practices that protect fish products from spoilage, adulteration, mishandling, and off- flavors. Processing waste can claim up to 70% by weight of finfish depending on the species and manner processed, and post-harvest losses can claim around 30%. Partnering with other groups and co-developing outreach techniques to reduce post harvest losses can significantly contribute to the amount of fish available for consumption; thus, contributing to the nutrition goals of USAID's Feed the Future Initiative. Certification, traceability, product integrity, and other efforts to improve fish products for consumer acceptance and international markets are desired. Gender integration is important to consider as women are strongly represented in the processing and marketing sectors, and throughout much of the value chains. **(**Aquaculture-Fisheries Nexus Topic Area)

Policy Development (PDV)
Policy initiatives that link aquaculture to various water uses to improve human health are needed. Areas of inquiry can include institutional efforts to improve extension related to aquaculture and aquatic resources management; science-based policy recommendations targeting poor subpopulations within a project area, or more broadly (for example, national aquaculture strategies); methods of improving access to fish of vulnerable populations including children (e.g., school-based aquaculture programs); science-based strategies for integrating aquaculture with other water uses to improve wellbeing, such as linkages with clean drinking water and improved sanitation. Additionally, social and cultural analyses regarding the impacts of fish farming may yield critical information for informing policy development.

Marketing, Economic Risk Assessment, and Trade (MER)
Aquaculture is a rapidly growing industry and its risks and impacts on livelihoods need to be assessed. Significant researchable issues in this arena include cost, price, and risk relationships; domestic market and distribution needs and trends; the relationships between aquaculture and women/underrepresented groups; the availability of financial resources for small farms; and the effects of subsidies, taxes, and other regulations. Understanding constraints across value chains in local, regional, and international markets is of interest, especially as constraints affect competitiveness, market demand, and how to link producers to specific markets. **(**Aquaculture-Fisheries Nexus Topic Area)

Watershed and Integrated Coastal Zone Management (WIZ)
Aquaculture development that makes wise use of natural resources is at the core of the AquaFish program. Research that yields a better understanding of aquaculture as one competing part of an integrated water use system is of great interest. The range of research possibilities is broad—from investigations that quantify water availability and quality to those that look into the social context of water and aquaculture, including land and water rights, national and regional policies (or the lack

thereof), traditional versus industrial uses, and the like. Water quality issues are of increasing concern as multiple resource use conflicts increase under trends toward scarcity or uneven supply and access, especially for freshwater. Ecoregional analysis is also of interest to explore spatial differences in the capacities and potentials of ecosystems in response to disturbances. Innovative research on maximizing water and soil quality and productivity of overall watersheds is of interest. Pollution is a huge concern, as over 50% of people in developing countries are exposed to polluted water sources. Additionally, aquatic organisms cannot adequately grow and reproduce in polluted waters, and aquaculture may not only be receiving polluted waters, but adding to the burden. Rapid urbanization has further harmed coastal ecosystems, and with small-scale fisheries and aquaculture operations in the nearshore, integrated management strategies for coastal areas are also important. **(**Aquaculture-Fisheries Nexus Topic Area)

Mitigating Negative Environmental Impacts (MNE)
With the rapid growth in aquaculture production, environmental externalities are of increasing concern. Determining the scope and mitigating or eliminating negative environmental impacts of aquaculture—such as poor management practices and the effects of industrial aquaculture—is a primary research goal of this program. A focus on biodiversity conservation, especially in biodiversity "hotspot" areas, as related to emerging or existing fish farms is of great interest. Therefore, research on the impacts of farmed fish on wild fish populations, and research on other potential negative impacts of farmed fish or aquaculture operations is needed, along with scenarios and options for mitigation. (Aquaculture-Fisheries Nexus Topic Area)

PROJECT STRUCTURE AND TECHNICAL CONSIDERATIONS

Investigations that build on existing work to generate new information, transfer information, and build in-country capacity form the core of AquaFish projects. The five 2016-2018 projects each contain four to six investigations, with a total of 24 investigations in AquaFish's current portfolio. While each investigation identifies a single topic area that best describes it, projects include multiple topic areas in describing aquaculture research that will improve diets, generate income for smallholders, manage environments for future generations, and enhance trade opportunities.

Each investigation is clearly identified as an experiment, study, or activity, with experiments involving hypothesis testing, studies resulting in the discovery of new information but not being hypothesis-driven, and activities including conferences, trainings, and other means for outreach and dissemination. This structure provides a transparent means for evaluating different types of work under AquaFish, be it quantitative, empirical, biologically-based, qualitative, policy-based, or informal.

To balance the portfolio of research and development with outreach and dissemination, each project includes at least one investigation identified as an experiment and at least one as an activity. In keeping with goals towards improving human health and gender equity, at least one investigation is focused on human health (assigned either the HHI or FSV topic area), and at least one investigation is focused on women and/or girls. These areas are combined in some cases, for example, as one activity focused on human health and women/girls.

In addition to the individual investigations focused on gender and outreach, each AquaFish project developed plans on outreach and dissemination and on gender inclusiveness that describe the project's comprehensive strategy for building capacity of HC researchers, farmers, and other stakeholders through improved understanding of aquaculture technologies; and for equitably involving women at all levels and stages of the project. Each project also has a plan for monitoring and evaluating its successes in reaching intended beneficiaries, with metrics specified through Feed the Future Monitoring System (FTFMS) indicator targets. Recognizing the importance of making federally funded data available to the public, and in compliance with USAID policies, each project developed a Data Management Plan that identifies anticipated data that will result from project work. Each project also laid out a strategy for a graceful exit once project funds end, describing plans for extending project outcomes beyond the life of the AquaFish Innovation Lab.

RESEARCH PRIORITIES

All five projects address the following general research priorities:

- **Priority Ecosystems -** Inland and coastal ecosystems for aquaculture and aquaculture-fishery nexus topic areas.
- **Priority Species -** Low-trophic level fishes; domesticated freshwater fishes; non-finfishes (e.g., bivalves, seaweeds); aquatic organisms used in polycultures and integrated systems; native species. Food fishes are a priority but species used for non-food purposes (e.g., ornamental, pharmaceutical) may also be included as a priority if they are a vital part of an integrated approach towards food security and poverty alleviation.
- **Target Groups -** Aquaculture farms (small- to medium-scale, subsistence, and commercial) and aquaculture intermediaries, policy makers, and others in host countries.
- **Key Partners -** Universities, HC and US government, NGOs, private sector, CGIAR Consortium (formerly Consultative Group on International Agriculture Research) and the USAID Food Security Innovation Center.

USAID also encourages AquaFish to address biodiversity conservation and non-GMO biotechnology solutions to critical issues in aquaculture. Each overall project describes a comprehensive development approach to a problem. Projects are formed around the following core program components in alignment with the AquaFish Lead Award from USAID: a systems approach; social, economic, and environmental sustainability; capacity building and institution strengthening; gender integration; high quality research with a pathway for outreach, dissemination, and adoption; food security with a focus on the poor; and climate change.

RULES OF CONDUCT

Rules of conduct are described in greater detail in each project's subcontract with the Management Entity and in other program documents. The following subset of rules is particularly relevant to the Implementation Plan.

Fostering Respectful Partnerships

Projects foster linkages with organizations including US minority-serving institutions, CGIAR, non-governmental organizations (NGOs), national agricultural research institutions, other USAID Food Security Innovation Labs, international centers, private businesses, and others are desired. Proposals that link HC researchers from one AquaFish site to another site are encouraged. US and HC PIs will share in budgetary decisions and overall priority setting for the project, as well as in other collaborative activities related to AquaFish. Proposals, work plans, and project budgets must be developed collaboratively between HC and US researchers. US PIs must actively establish and maintain an effective working relationship with the Director and Management Team, and other AquaFish US and HC project PIs and program participants.

Memoranda of Understanding

Within 30 days of award notification, the Lead US Institution of each project is required to enter into Memoranda of Understanding (MOUs) and/or subcontracts with institutions at HC sites. MOUs and subcontracts with existing institutions will need to be renewed and filed with the Management Office

(MO). Subcontracting US institutions may also enter into MOUs with HC partners to strengthen institutional relationships and streamline administrative processes. MOUs must provide the opportunity for other AquaFish projects to function under the authority of the agreement and must provide for joint authorship of reports and site visits at the discretion of the AquaFish Management Entity. Draft MOUs with new institutions are submitted to the MO for review prior to execution.

Environmental Compliance

The following USAID environmental restrictions apply to the projects and the overall program:

- Biotechnical investigations will be conducted primarily on research stations in Host Countries;
- Research protocols, policies and practices will be established prior to implementation to ensure that potential environmental impacts are strictly controlled;
- All training programs and outreach materials intended to promote the adoption of AquaFish-generated research findings will incorporate the appropriate environmental recommendations;
- All sub-awards must comply with environmental standards;
- AquaFish Projects will not procure, use or recommend the use pesticides of any kind. This includes but is not limited to algicides, herbicides, fungicides, piscicides, parasiticides, and protozoacides;
- AquaFish Projects will not use or procure genetically modified organisms (GMO); and
- AquaFish Projects will not use or recommend for use any species that are non-endemic to a country or not already well established in its local waters, or that are non-endemic and well established but are the subject of an invasive species control effort.

Fund Matching and Distribution

At least 50% of funds must be expended in the Host Country or region. Each project must supply an additional 25% of US non-federal matching funding from participating US entities and target 25% from participating HC institutions. Collaborative efforts that involve undergraduate students, graduate students, and post-doctoral fellows are encouraged. AquaFish funds will not be used to support US expatriate personnel or consultants, as the Innovation Lab model is intended to build institutional networks and capacities. In furtherance of the Title XII initiative that authorizes all Innovation Labs, projects must demonstrate return benefits to the US. Under Title XII, AquaFish has responsibility to provide mutual benefits and discoveries that can apply to the HC region and US, and that will support future development of sustainable aquaculture and fisheries.

PART I. RESEARCH PROJECT SUMMARIES

ASIA PROJECT: BANGLADESH

❖

Project Title

ENHANCING AQUACULTURE PRODUCTION EFFICIENCY, SUSTAINABILITY AND ADAPTIVE MEASURES TO CLIMATE CHANGE IMPACTS IN BANGLADESH

AquaFish Project Theme

ENVIRONMENTAL MANAGEMENT FOR SUSTAINABLE AQUATIC RESOURCES USE

Investigations

16SFT02NC	Nutritional Conditioning During Larval Development to Improve Feed Efficiency and Identify Beneficial Gut Flora in Tilapia
16IND02NC	Tilapia and Koi (Climbing Perch) Polyculture with *Pangasius* Catfish in Brackish (Hyposaline) Waters of Southern Bangladesh
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
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
16MNE02NC	Dissemination of AquaFish Innovation Lab Technologies for Improving Food Production Efficiency and Livelihoods of the People of Bangladesh

US and Host Country Institutions

US	North Carolina State University (Lead US Institution)
Bangladesh	Bangladesh Agricultural University (Lead HC Institution)
	Khulna University
	Patuakhali Science and Technology University
	Shushilan NGO

Project Summary

Bangladesh, one of the most densely populated countries in the world, has high rates of poverty and widespread malnourishment, particularly among women and children. Sustainable aquaculture in Bangladesh is one solution for increasing food security, augmenting dietary nutrition, and improving the economic livelihoods for its poorest citizens. However, technical, environmental, and economic barriers limit aquaculture production in the country. This project, through five investigations, seeks to address such barriers by developing technologies for enhancing aquaculture production efficiency, intensification, and sustainability to improve household income and nutrition, particularly for low-income farming households. With the hope of increasing feed efficiency and reducing associated costs, researchers will evaluate the effectiveness of nutritional conditioning and characterize the respective changes in gut microbial communities and nutrient absorption in tilapia. To address environmental and economic concerns for the existing farming industries of shrimp and prawn, researchers will continue to assess the potential for farming *Pangasius* catfish in brackish (hyposaline) waters in regions traditionally reliant solely upon shrimp farming. Researchers will also examine a novel polyculture/land-farming strategy, culturing Mola (*Amblypharyngodon mola*) with prawns and using pond muds as fertilizer to grow fresh vegetables on unflooded gher-dykes. Lastly, researchers will evaluate the effects of reducing feed and investigate polyculture technology, particularly in indigenous, air-breathing fishes, such as Shing and Koi (nutrient rich and hardy fish), to enhance incomes and dietary nutrition, while reducing environmental impacts. To maximize the adoption and impact of improved technologies, researchers will hold a series of workshops and trainings, complemented by the distribution of outreach materials.

ASIA PROJECT: CAMBODIA & VIETNAM

❖

Project Title

IMPROVING FOOD SECURITY, HOUSEHOLD NUTRITION, AND TRADE THROUGH SUSTAINABLE AQUACULTURE AND AQUATIC RESOURCE MANAGEMENT IN CAMBODIA AND VIETNAM

AquaFish Project Theme

ENHANCED TRADE OPPORTUNITIES FOR GLOBAL FISHERY MARKETS

Investigations

16SFT01UC	Pellet Feed Improvements Through Vitamin C Supplementation for Snakehead Culture
16IND01UC	Sustainable Snakehead Aquaculture in Cambodia
16QSD01UC	Genetic Diversity of Striped Snakehead (*Channa striata*) in Cambodia and Vietnam
16FSV01UC	Enhancing Food Safety and Household Nutrition of Women and Children Through Aquaculture and Capture Fisheries in Cambodia and Vietnam in the Dry Season
16PDV01UC	Guidance and Policy Recommendations for Sustainable Snakehead Aquaculture and Aquatic Resource Management in Cambodia and Vietnam

US and Host Country Institutions

US	University of Connecticut – Avery Point (Lead US Institution)
	University of Rhode Island
Cambodia	Inland Fisheries Research and Development Institute (Lead HC Institution)
Vietnam	Can Tho University

Project Summary

The productive Mekong fisheries are essential to the food security and nutrition of the 60 million people of the Lower Mekong Basin. Fish, from capture and culture, are a significant source of income and food security in Cambodia and Vietnam. The rapid growth of freshwater aquaculture in both countries represents an opportunity to improve the livelihood of their residents. This project builds on past AquaFish work through five integrated investigations that support the development of sustainable aquaculture, enhancement of trade, and improvement of aquatic resource management, with a focus on sustainable snakehead aquaculture after the ban of snakehead farming was lifted in Cambodia in April 2016. To address the sustainability of the popular snakehead industry, researchers will work to develop alternative cost-effective feeds, compare growth performance and survival rate of different snakehead strains, improve value-added processing techniques typically undertaken by women, conduct an economic assessment, and develop policy recommendations for sustainable snakehead production. A household survey will explore the availability of fish, as well as perceived versus actual benefits of consuming fish. The results of these efforts will inform strategies and policies that address nutritional deficits, particularly for women and children in Cambodia.

AFRICA PROJECT: GHANA & TANZANIA

❖

Project Title

AQUACULTURE DEVELOPMENT AND THE IMPACT ON FOOD SUPPLY, NUTRITION, AND HEALTH IN GHANA AND TANZANIA

AquaFish Project Theme

IMPROVED HUMAN HEALTH AND NUTRITION, FOOD QUALITY, AND FOOD SAFETY

Investigations

16BMA01PU	Experimental Pond Unit Assessment in Tanzania
16BMA02PU	Optimizing the Use of Commercial Feeds in Semi-Intensive Pond Production of Tilapia in Ghana; From Nursery to Grow-Out
16SFT03PU	Increasing Productivity of Nile Tilapia (*Oreochromis niloticus*) Through Enhanced Feeds and Feeding Practices
16HHI02PU	Fish Consumption and Implications for Household Nutrition and Food Security in Tanzania and Ghana
16MER01PU	Enhancing the Functionality and Applicability of Fish Market Information System (FMIS) to Marine Artisanal Fisheries in Ghana

US and Host Country Institutions

US	Purdue University (Lead US Institution)
	Virginia Polytechnic Institute and State University
	University of Arkansas at Pine Bluff
Ghana	Kwame Nkrumah University of Science & Technology (Lead HC Institution)
Tanzania	Sokoine University of Agriculture

Other Collaborations and Linkages
Farmer Line (Ghana)

Project Summary

In sub-Saharan Africa, fish is an important source of protein, essential micronutrients, and minerals in the diet of most households. Thus, fish and their sustainable production are major contributors to food security and improved livelihood in Ghana and Tanzania. However, supply of fish is currently low, causing limited consumption levels. Through five investigations, this project builds on previous AquaFish work to enhance the various facets of aquaculture and its contribution to food supply, nutrition, and health in Ghana and Tanzania. The cost of quality feed frequently limits aquaculture production; hence, researchers continue working to develop cost-effective diets from locally available ingredients (e.g., earthworm, maggot meals) and evaluate the profitability of such feeds in comparison to commercial feeds. To train educators, students, and fish farmers in determining better methods of feeding, fertilizing, and managing water quality, the project will compare fertilization and feeding strategies and evaluate the physical, chemical, and biological characteristics of ponds during grow-out. To better inform and empower stakeholders along the fish-value chain and more efficiently support markets, researchers will train farmers and fishermen on the use of a cell-phone-based information system and broaden its applicability to include marine fisheries. Through a household survey on dietary diversity and an analysis of household consumption practices, researchers plan to formulate policy measures that improve aquaculture and fisheries practices in order to increase household food security

ASIA PROJECT: NEPAL

❖

Project Title

ADVANCING AQUACULTURE SYSTEMS IN NEPAL FOR MORE SOCIAL AND ENVIRONMENTAL SUSTAINABILITY

AquaFish Project Theme

ENVIRONMENTAL MANAGEMENT FOR SUSTAINABLE AQUATIC RESOURCES USE

Investigations

16BMA03UM A comparison of monoculture and polyculture of tilapia with carps for pond production systems in Nepal

16BMA04UM Developing new systems for periphyton enhancement in farmers' ponds

16QSD02UM Improving seed production of sahar (*Tor putitora*) in Chitwan, Nepal

16HHI03UM Outreach to increase efficiency of aquaculture in Nepal

US and Host Country Institutions

USA University of Michigan (Lead US Institution)
Nepal Agriculture and Forestry University (Lead HC Institution)

Other Collaborations and Linkages
Fisheries Research Center, Nepal Agricultural Research Council
Directorate of Fisheries Development

Project Summary

Nepal is a poor country with many undernourished or even malnourished residents and low levels of education, with primary schooling being the highest level of education for most residents. As a result of this poverty, most planning documents produced by the government, as well as outside organizations, concentrate on human health and nutrition as the main focus for future development of aquaculture in this region. The primary focus of this project is to improve food security and nutrition for rural communities in Nepal through a suite of investigations on small-scale aquaculture that consider environmental management for sustainable aquatic resources use. Outreach programs for rural farmers and their families will be continued, as well as expanding school ponds for education of rural youth and women's groups. To improve efficiency of production systems and reduce effluents discharged during harvest, researchers are exploring ways to enhance periphyton growth in existing ponds. Production efficiency is also being addressed through an investigation on the polyculture of carp with Nile tilapia (*Oreochromis niloticus*) and sahar (*Tor putitora*). Researchers will also focus on enhancing the production of native fish species, with particular attention to sahar, an economically important, high-valued indigenous fish species in Nepal. By incorporating sahar in on-farm trials for improving culture techniques, researchers hope to expand seed production, a major bottleneck to aquaculture development in Nepal.

AFRICA PROJECT: KENYA & UGANDA

❖

Project Title

AQUACULTURE DEVELOPMENT IN KENYA AND UGANDA: ADVANCING COST-EFFECTIVE TECHNOLOGY, MARKET ASSESSMENT, AND END-USER ENGAGEMENT

AquaFish Project Theme

INCOME GENERATION FOR SMALL-SCALE FISH FARMERS AND FISHERS

Investigations

16IND03AU	Development of Low-Cost Captive Breeding and Hatching Technologies for the African Lung Fish (*Protopterus aethiopicus* and *P. amphibius*) to Improve Livelihoods, Nutrition, and Income for Vulnerable Communities in Uganda
16FSV02AU	Implementing and Assessing Cell-Based Technical and Marketing Support Systems for Small and Medium-scale Fish Farmers in Uganda
16MER02AU	Assessment of Price Volatility in the Fish Supply Chain in Uganda
16HHI04AU	Women in Uganda Aquaculture: Nutrition, Training, and Advancement
16BMA05AU	Water, Water Quality, and Pond Bottom Soil Management in Ugandan Aquaculture

US and Host Country Institutions

USA	Auburn University (Lead US Institution)
	North Carolina State University
	Alabama A&M University
Kenya	University of Eldoret
Uganda	Makerere University (Lead HC Institution)

Other Collaborations and Linkages
National Fisheries Resources Research Institute
Fisheries Training Institute

Project Summary

Poor families in developing countries typically spend 50 to 70 percent of their income on food. When quality food becomes too expensive, women tend to modify their consumption, often turning to cheaper alternatives that lack in essential nutrients. To increase income and reduce the prevalence of undernutrition, enhancing access to fish and sustainable aquaculture is important. This project will build on previous AquaFish achievements to address obstacles to the development and growth of aquaculture in Uganda and Kenya, such as working with industry to ensure the supply of quality seed and feed to enable income generation for small-scale fish farmers. Researchers will develop low-cost captive breeding and hatching technologies of African lungfish (*Protopterus aethiopicus* and *P. amphibius*) that will introduce new opportunities for farming popular native species that are less vulnerable to a changing climate than many non-native species. To increase income for fish farmers and improve (and expand) markets of farmed fish, researchers will assess price volatility in the fish supply chain in Uganda, in addition to creating a cell-phone network that will connect people throughout the aquaculture value chain. With a goal of mitigating negative environmental impacts of aquaculture, researchers will measure various metrics of water quality in farmed water-bodies and evaluate the need for water quality amendments. This project will also train and support women in aquaculture. With the help of institutional partners and industry, researchers will hold a series of capstone events, including the annual fish farmer symposium in Uganda, that will train women on the nutritional value of new species and improve women's access to information about the entire value chain of aquaculture.

PART II. RESEARCH PROJECT INVESTIGATIONS

TOPIC AREA

PRODUCTION SYSTEM DESIGN AND BEST MANAGEMENT ALTERNATIVES

❖

EXPERIMENTAL POND UNIT ASSESSMENT IN TANZANIA

AFRICA PROJECT: GHANA & TANZANIA
US Project PI: Kwamena Quagrainie, Purdue University
HC Project PI: Steve Amisah, Kwame Nkrumah University of Science & Technology

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

Collaborating Institutions and Lead Investigators

Virginia Polytechnic Institute and State University (USA)	Emmanuel Frimpong
Sokoine University of Agriculture (Tanzania)	Sebastian W. Chenyambuga
	Nazael Madalla
	Hieromin Lamtane
Oregon State University (USA)	Hillary Egna
University of Michigan (USA)	James S. Diana
Kwame Nkrumah University of Science & Technology (Ghana)	Daniel Adjei-Boateng
	Nelson W. Agbo

Objectives

1. To evaluate ponds in Tanzania for their physical, chemical, and biological characteristics during a grow-out.
2. To compare the effects of fertilization alone, full ration feeding alone, and a combination of fertilization and feeding at 50% satiation on growth performance and production yield of Nile tilapia.

Significance

Aquaculture is considered an option for rural development because it can help to solve problems of protein malnutrition and income poverty and provide employment to rural poor people. In Tanzania, aquaculture is an emerging industry and dominated by pond culture of Nile tilapia (*Oreochromis niloticus*). The industry is dominated by small-scale farmers producing fish for household consumption and for the domestic market. However, fish production in many small-scale ponds is low due to low productivity of the commonly cultured species coupled with poor management. Given the importance of aquaculture in the country, there is a need to improve fish production to complement the declining capture fisheries. Improvement in aquaculture production can enhance food security directly by producing fish for household consumption and by improving the supply and reducing the price of fish in the market (Jahan et al., 2010). Fish consumption has been shown to improve the nutritional status and lower prevalence of malnutrition in rural poor households (Aiga et al., 2009). Fish farming contributes to improvement of wellbeing of poor people indirectly through diversification of sources of income and creation of new employment opportunities. With increased household income from fish farming activities, households are also able to purchase animal based foods with better sources of micronutrients needed by the household members, especially children (Aiga et al., 2009).

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. Studies have shown that proper pond fertilization combined with supplementary feeding at 50% satiation level results in higher fish yield and benefit-cost ratio (Wahab et al., 2014; Phanna et al., 2014).

This pond characterization experiment has two goals; the first is to evaluate ponds at each research site for their physical, chemical, and biological characteristics during a grow out, and the second is to compare the effects of fertilization alone, full ration feeding alone, and a combination of fertilization and feeding at 50% satiation on growth performance and production yield of Nile tilapia. The methods for pond characterization are well described in a number of publications, including Egna et al. (1987) and the *Standard Methods for the Examination of Water and Waste water* (multiple versions of this are available; the most recent is APHA et al., 2012). The purpose of this document is to describe a series of measurements that will be carried out for the AquaFish Innovative Lab, and to outline some of the reasons for these measurements.

Physical characteristics

A number of physical characteristics are important in aquaculture, particularly for the completion of quality experiments in ponds. The most obvious ones include pond morphometry, pond depth, evaporation rate, seepage rate, and water temperature.

Morphometry, or the physical shape of the pond, is important because it describes the physical place where experiments occur, and helps to assess water loss and replacement in volume. Usually morphometry is measured from the pond banks, and the actual working pond morphometry then is dependent on how much water is added to the pond. Morphometry can most easily be measured by simply making a number of transects across the pond itself, from the bank top, and measuring the depth at regular intervals (usually some frequency like 10 per transect or every meter) across the transect. In this manner, the surface area and pond volume can be calculated at each depth for the working pond.

Pond depth is a simpler measure that describes the amount of water in a pond at any time. It is usually measured at the deepest point in a pond, which is often either the center or the depth at the drain or monk. The measures of pond depth are usually taken daily, and water is often replaced on a weekly basis to maintain a regular pond depth. Pond depth is also controlled during a rain event by the drain stand pipe or monk boards, which only allow water to reach a certain level before it is discharged. For our work, depth will be measured and water replaced weekly, if necessary. Also, it is important to measure the loss of water from a pond during rainfall events, as this water will carry nutrients and other materials.

Evaporation of water occurs continually from a pond, depending on humidity, temperature, and wind. We can measure evaporation rate simply with an evaporation pan, and relate it to the other physical parameters mentioned above. Typically, evaporation is not measured regularly in ponds, in most cases only 2-3 times per grow out. For our case, that would be at stocking, mid grow out, and harvest.

Seepage is a similar measure that takes into account the water leakage from a pond. It does vary with pond depth, and again it strongly affects water use in aquaculture. Once again, seepage can be measured by evaluating changes in pond depth and those changes expected to occur due to evaporation. We should measure seepage at the same time that we measure evaporation rate, to simplify the calculations.

Finally, water temperature is the last physical parameter to be measured. This is complicated by the fact that ponds are shallow and exposed to sunlight and wind. Therefore, they may warm dramatically during the day, may stratify on calm days, and may mix thoroughly on windy days or at night. Hence, temperature must be measured several times at multiple depths to get relevant information. The existence of data sondes that can monitor temperature and oxygen over time have simplified this process, and it would be most relevant to do continual measurements at about hourly intervals at multiple depths, at least including surface, middepth, and just off the bottom. The measures can provide a good overview of the temperature characteristics influencing the pond, and also can be used to determine rates of primary production in the pond. Finally, because seasonal events also strongly influence temperature, and because it is an important factor influencing animal physiology, we should also measure temperature at three depths on weekly basis.

Chemical Parameters

The chemistry of pond water has dramatic effects on the pond ecosystem as well as the organisms being cultured. It is also an indicator of management methods and their success. While a very large number of variables can be monitored, in our pond experiments we will focus on those most commonly related to production in a pond. These include dissolved oxygen, phosphorus, various forms of nitrogen, pH, alkalinity, and dissolved and suspended solids. For marine or brackish water systems, salinity would be another important factor.

Dissolved oxygen (DO), like temperature, can stratify dramatically in ponds and also changes dramatically over the diel period. Maximum DO occurs during the day in ponds with much primary production, and the minimum occurs at dawn after a long night of respiration alone. Also, if pond water stratifies, then most likely oxygen will stratify even more strongly than temperature. This is particularly true in shallow ponds as most commonly light penetration is limited to the upper 40 cm or so of water, and all primary production occurs in this zone, while in deeper waters, even during the day, limited oxygen may be produced and oxygen levels may decline from the time of first stratification until the pond is mixed again in the evening. Therefore, like temperature, we should measure DO on an hourly basis and at various depths, again most reasonably the surface, midwater, and bottom for ponds of depths around 1 m. The simplest method for this is to use a data sonde that can continually measure and record DO and temperature. If this is not available, then a DO meter can be used and either set up for continual measures or manually used each hour at each depth needed. Since DO is also a very important parameter for survival of animals in ponds, we should also measure it regularly at dawn to evaluate longer term trends. These measures should be done on a weekly basis.

In freshwaters, phosphorus is considered the major limiting nutrient. In such waters, addition of phosphorus in the form of triple super phosphate can result in increased rates of primary and secondary production, and depending on the species present, increased production of the target organisms. While phosphorus is found in several forms, most commonly we have measured the total phosphorus concentration of pond water since the conversion between these forms tends to occur very rapidly. While phosphorus can vary over the day or at depth, it is not so dramatic as DO and temperature. Therefore, we most commonly measure total phosphorus during midday using a mixed water column sample. This mixed sample would include water from all depths of the pond, and is usually collected with a large pipe that can be lowered and sampled to include all depths in the column. The measurement frequency should be weekly.

If phosphorus is the limiting nutrient in pond water, then its addition will increase primary production. However, at some level phosphorus will become available in surplus, and then no further increase in primary production will occur with additional inputs. If regular water quality measurements were being made, this point would be obvious by phosphorus increasing in concentration in the water column. At this point some other nutrient has become limiting, and most likely this will be nitrogen. Addition of nitrogen, in the form of urea, nitrate, and the like can further stimulate primary production. Combined supplementation of nitrogen and phosphorus will then continue to drive up even higher rates of primary production. Pond experiments in Thailand have shown that the optimum rate of fertilization is 4 kg N and 1 kg P per ha per day. At this optimum rate, both nitrogen and phosphorus are input at rates that allow high rates of primary production yet do not result in drastic declines in DO and give high production rates for Nile tilapia.

Nitrogen in pond water varies in form depending on the nitrogen cycle. Depending on the aerobic nature of the pond water, and on pH, some of these forms can be toxic while others are necessary for primary production. For these reasons, all forms of nitrogen are monitored in the pond. Nitrate is the most readily taken up by plankton for photosynthesis, and is often the dominant form of nitrogen in the water. Ammonia (NH_3) is given off as a waste product by aquatic organisms. It can be toxic when it is converted back to ammonium (NH_{4+}) at high water pH. Nitrite (NO_2) is an intermediate form of nitrogen in the nitrogen cycle as it is converted from NH_3 back to NO_3, and can also be toxic to animals at high pH. Finally, total Kjeldahl nitrogen (TKN) is the nitrogen contained dissolved in the water as well as that found in microorganisms in the water column. Dissolved inorganic nitrogen is the sum of NO_2, NH_3, and NO_3. In our pond characterization, we will measure all of these forms, including NO_2/ NO_3, NH_3, and TKN. Since nitrogen forms do not show large changes with depth or time of day, they will also be measured weekly using a composite water sample around midday.

pH is the measure of acidity in the water. It is affected by a large number of chemical characteristics, including the water source, the balance of carbon in the water, and other acids or bases in solution. It also can show variations with depth and with time, as it is affected by forms of carbon in the pond and therefore by rate of primary production, at least in waters with moderate to low levels of alkalinity. Therefore, we measure pH hourly at each depth, as it is done with DO and temperature. Data sondes often have pH as an added variable measured, making this a fairly simple measure. Otherwise, manual measurement with a pH meter will be necessary. Since pH influences toxicity of NO_2 and NH_3, we should also measure it weekly on a composite sample.

Alkalinity is a measure of the combination of carbon in the water. It includes carbon in the forms of carbon dioxide, carbonate, and bicarbonate. With more dissolved carbon in the water, usually the water becomes more basic in pH, and therefore the measure of alkalinity is usually done by titrating water to a set level of pH. Highly alkaline waters have lots of carbon for primary production, and therefore are usually limited by phosphorus, nitrogen, or light penetration. However, low alkalinity waters, particularly those below 30 mg/L alkalinity as $CaCO_3$, may become limited in carbon as well. If feed or organic fertilizers are added to pond waters, they can increase alkalinity because they have lots of carbon contained within. However, if just inorganic forms of TSP and NH_3 are used for fertilization, then alkalinity may decline and carbon become limiting to primary production. While we can simply measure alkalinity once a day to get a reasonable idea of the carbon conditions in a pond, if we use the changes in pond water to determine primary production rates, then we should measure alkalinity on a diel basis similar to temperature, DO, and pH. Hence, both weekly and diel measures are appropriate.

The final chemical variable for freshwater systems is a measure of solids. Total dissolved solids (TDS) include many of the elements listed before, plus others. Total Suspended solids (TSS) are those that are in the water column usually due to the source water, but not dissolved. Suspended solids cause turbidity, and can limit primary production by reducing light penetration. While many components of suspended solids will settle out of supply water if it is held in a calm state, some colloids of clay will remain in solution and raise turbidity. In any case, both TSS and TDS should be measured on a weekly basis like phosphorus and nitrogen.

Biological characteristics

Besides the biomass and production of the target organisms in a pond (which we are not measuring in this experiment), other biological characteristics are important. Generally, the interest would be the amount of phytoplankton production in ponds, either by estimating the rate of primary production or the phytoplankton standing crop. While bacteria and other microbes may be very important in pond culture, we have not regularly measured microbial processes in pond waters.

Phytoplankton in the water may be characterized by their species composition, but this is a tedious process and usually not undertaken unless an experimental protocol is particularly interested in the production of certain phytoplankton species. However, total plankton biomass is a variable of interest to most pond culture systems. We can estimate plankton standing crop by measuring light penetration. Since algae in pond water block light penetration, the lower the light penetration, the higher the plankton standing crop. Of course, solids in the water can also influence light penetration, and the best comparisons would be when changes in light penetration are measured over time in a water system. The simplest measure of light penetration is the Secchi disk, which is lowered into the water until it disappears from sight. The amount of light penetration is twice the Secchi disk depth, since for one to see light reflection from the disk, light has to penetrate both down to the disk and back up to the eye. Usually a measure of 2xSDD is considered the compensation point or the depth at which sufficient light penetrates to allow for primary production. Thus, in this case a shallow SDD indicates high concentration of plankton, and a deep SDD indicates less plankton biomass. Since biomass is often related to production, low SDD may also indicate high rates of primary production, although this measure is less directly proportional than the relationship to biomass.

Another measure of plankton biomass is the concentration of chlorophyll in the water column. Since chlorophyll is present in all photosynthesizing algae, it is an indicator of algal biomass. Again, we typically measure chlorophyll-a because it is the dominant form of chlorophyll, and measure it on a weekly basis from combined water samples.

Primary production is a measure of the rate of photosynthesis. Historically, it was measured by the light-dark bottle method, and the increase in DO in a bottle of water with light allowed to penetrate was used as an estimate of net primary productivity (photosynthesis minus respiration in the organisms contained in the bottle), while the decline in DO in the bottle kept in the dark was a measure of respiration only, and the sum of respiration plus NPP gave the gross primary production of the organisms in the bottle. This works well for low productivity, natural waters where it may take several days to measure an increase in the light bottle and a decline in the dark bottle. In highly eutrophic aquaculture ponds, the high amounts of material added result in high rates of respiration, and dark bottle DO often declines to near zero in a matter of hours. Similarly, since light penetration and primary production are related to depth and stratification in ponds, a number of bottles at different depths would be necessary to reasonably approximate whole pond production. Several studies have shown that the light-dark bottle method is not suitable for estimating primary production in aquaculture ponds.

A second method which is preferable as an estimate of primary production in aquaculture ponds is the whole pond method. This method uses the increases in DO during the day and the declines at night to approximate photosynthesis and respiration in the pond. Of course, diffusion at the pond surface may also influence DO levels, so it may be necessary to correct for diffusion in the estimate. Once again, since the values of DO, pH, alkalinity, and temperature are being collected at 3 depths and on a regular basis over the diel period; we can use these to calculate the net primary productivity, respiration, and gross primary productivity of the whole pond system. Templates for these calculations are available in several CRSP documents. For a reasonable estimate of whole pond production, measurements of DO, temperature, pH, and alkalinity should be taken at dawn, midday, dusk, midnight, and the next dawn.

Summary
This document lists the main water quality variables to be monitored in pond aquaculture, and the reasons for the number and frequency of those measurements. A summary table below lists the variables again and their metrics. Methods for the measurement of each parameter are described in Egna et al., (1987) and are partly based on APHA et al., (2012).

Quantified Anticipated Benefits
The completion of a pond characterization experiment in Tanzania will provide a complete understanding of the strengths and weaknesses of IAAS for further experiments. Through this understanding improvements in the infrastructure and training of project personnel could be done if necessary to fulfill future research. In addition, a better understanding of the physical, chemical, and biological characteristics of ponds in Tanzania will aid culturists in determining better methods of feeding, fertilizing, and managing water quality. This could lead to increased profits, the supply of high quality fish protein to communities with limited food resources, and the overall growth of the aquaculture sector. The expected deliverables are as follows:
1. Better management strategies for pond fertilization and fish feeding determined and promoted;
2. Data for physical, chemical, and biological characteristics of ponds established for future AquaFish innovative lab experiments;
3. At least 40 fish farmers trained through training workshops and adopt better pond fertilization and fish feeding strategies;
4. At least five MSc students trained on pond water quality management strategies.

Research Design and Activity Plan
The experiment will be conducted in 12 earthen ponds, each with the size of 100 m^2. There will be three treatments and each treatment replicated four times. Treatment 1 (T_1) will be the control involving four ponds receiving fertilization alone. Treatment 2 (T_2) will involve four ponds with feed applied *ad libitum*, (only feeding with no fertilizer application). Treatment 3 (T_3) will involve four ponds with a combination of both feeding and fertilizer application, feeding will be at half satiation (fertilization plus supplementary feeding at 50% satiation level). The treatments will be randomly allocated to the experimental ponds in a completely randomized design.

Location
The study will be conducted at the Aquaculture Research Facility, Department of Animal Science and Production, Sokoine University of Agriculture.

Methods
Before the start of the experiment water will be drained from all ponds. The ponds will be left empty for a period of 14 days to allow them to dry. After drying, agricultural limestone ($CaCO_3$) will be applied to the pond bottom and the slopes of the dykes at a rate of 100 g/m^2 and after seven days the ponds will be filled with water to a depth of 1 m. All ponds will be stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) at a stocking density of 2 $fish/m^2$. Fertilization will commence one week before stocking. Fertilization will be at 4 kg N and 1 kg P per hectare daily, using urea and Diammonium phosphate (DAP), and will be applied once a week. Feeding will be done twice daily, in the morning (10.00 am) and in the evening (16.00 pm). Locally available feeds will be used and the diet will comprise of wheat bran (50%), fish meal (25%), cotton seed cake (10%), sunflower seed cake (11%) and maize meal (4%). The diet will be formulated to contain 30% CP.

Pond water level will be maintained at a depth of 1 m by topping up weekly to replace losses due to seepage and evaporation. Ponds will be managed for 120 days, and then harvested. Weekly measurements of water quality parameters as described above and also in Table 1 will be done in mid-week from water collected at the center of the pond. Diel measurements will be made biweekly up to the end of the experiment using the methods described above. Temperature in ^{0}C and dissolved oxygen mg l^{-1} will be measured by using YSI 55 dissolved oxygen meter (model 55, Yellow Spring Instrument Co. Ohio, USA). Water pH will be measured using a pH meter (Mardel 5, R029B-MARDEL, USA). Total alkalinity, nitrate-nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N), total dissolved solid (TDS), conductivity, and phosphate-phosphorus (PO_4-P) will be determined using HACH Kit (DR - 4000, a direct reading spectrophotometer). The transparency of water will be measured using a secchi disc of 20 cm diameter. Chlorophyll-a will be determined spectrophotometrically following GF/C glass fiber filtration and acetone extraction procedure.

Body weight and length of individual fish in each pond will be weighed at the start of the experiment to determine initial weight and length, respectively, and then every two weeks up to the end of the experiment. Body weight will be weighed in gram using a sensitive weighing balance and body length will be measured in cm using a measuring ruler. Death of fish will be recorded every day. At the end of the experiment, fish will be harvested by repeated netting using a seine net and then by de-watering the ponds using low-lift pump. Body weight and length of harvested fish will be weighed to determine final body weight and length, respectively.

Data including yield, growth rate, and survival will be computed for fish from all treatments, and comparisons made using analysis of variance (ANOVA). Differences in water quality between treatments and over time will be tested using ANOVA. In addition to chemical concentrations, diel measurements will be used to determine stratification in the ponds and primary productions rates. These will also be compared among treatments using ANOVA.

Table 1. Physical, chemical, and biological characteristics to be sampled during pond characterization.

Variable	Daily Frequency	How often	Type of sample
Pond morphometry	-	Once	
Pond depth	Once	Daily	One
Evaporation	Once	biweekly	One
Seepage	Once	biweekly	One
Temperature	Diel measures	biweekly	3 depths
Dissolved oxygen	Diel measures	biweekly	3 depths
pH	Diel	biweekly	3 depths
Alkalinity	Diel	biweekly	3 depths
Water depth	Once	Weekly	Whole pond
Temperature	Once	Weekly	3 depths
DO	Once	Weekly at dawn	Composite sample
pH	Once	Weekly	Composite sample
Alkalinity	Once	Weekly	Composite sample
Total phosphorus	Once	Weekly	Composite sample
Total Kjeldahl nitrogen	Once	Weekly	Composite sample
Ammonia	Once	Weekly	Composite sample
NO3/NO2	Once	Weekly	Composite sample
Secchi disk depth	Once	Weekly	Whole pond
Chlorophyll-a	Once	Weekly	Composite sample

<u>Trainings and Deliverables</u>

- Five MSc students will be involved in this research project as one way of long-term training and capacity building on assessment of pond dynamics.
- One workshop will be conducted for fish farmers and Village Extension Officers to train them on water quality management. The target is to attract 40 participants from at least four villages.

- The project will be gender sensitive and it will ensure that 50% and 10% of the farmers attending the training are females and youth, respectively.

Schedule

	2016						2017											
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Activity 1.1: Draining of water and drying of experimental ponds	■																	
Activity 1.2: Liming of experimental ponds	■																	
Activity 1.3: Filling water and fertilization of experimental ponds	■																	
Activity 1.4: Stocking of fingerlings	■																	
Activity 1.5: Weekly fertilization of experimental ponds	■	■	■	■	■	■												
Activity 1.6: Daily feeding of fish	■	■	■	■	■	■												
Activity 1.7: Data collection by measuring water quality parameters	■	■	■	■	■	■												
Activity 1.8: Data collection by measuring fish body weight and length	■	■	■	■	■	■												
Activity 1.9: Fish harvesting and determination of production yield						■												
Activity 1.10: Data analysis and report writing						■	■											
Activity 1.11: Training workshop								■	■	■		■						■
FINAL REPORT WRITING							■					■						■

OPTIMIZING THE USE OF COMMERCIAL FEEDS IN SEMI-INTENSIVE POND PRODUCTION OF TILAPIA IN GHANA; FROM NURSERY TO GROW-OUT

AFRICA PROJECT: GHANA & TANZANIA
US Project PI: Kwamena Quagrainie, Purdue University
HC Project PI: Steve Amisah, Kwame Nkrumah University of Science & Technology

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

Collaborating Institutions and Lead Investigators

Virginia Polytechnic Institute and State University (USA) — Emmanuel Frimpong
Kwame Nkrumah University of Science and Technology (Ghana) — Steve Amisah, Regina Edziyie, Nelson W. Agbo, Daniel Adjei-Boateng, Kwasi Obirikorang
University of Arkansas at Pine Bluff (USA) — Rebecca Lochmann
Purdue University (USA) — Kwamena K. Quagrainie

Objectives

1. To evaluate the survival, feed conversion, and growth of Nile tilapia raised in ponds on high-protein 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 from commercial feeds in combination with fertilization.
3. To compare growth and yield of Nile tilapia in grow-out ponds with 30% protein from commercial feeds at full and 50% 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 fingerlings.
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 phytoplankton and zooplankton composition and succession under objectives 1 and 5.
7. To evaluate the profitability of the different feeding strategies based on the outcomes of the experiments in objectives 1, 2, 3 and 5.

Significance

Fish account for over 50% of total animal protein consumed in most developing countries (FAO, 2007). Fish and other aquatic animals make a significant contribution to food and nutrition security in many Asian and African countries, where large numbers of people are poor and undernourished (Kent, 1987; World Bank, 2006). In developed countries fish consumption is increasing because of associated health benefits. Fish is by far the most frequently consumed animal source of food consumed in Asia and Africa (Gomna and Rana, 2007; Hortle, 2007; Bell et al., 2009; Biederlack and Rivers, 2009 and Belton et al., 2011). Aside from being a means to secure nutritional gains and of income generation, poverty reduction and food security, aquaculture is increasingly becoming a means to increase domestic fish supply to low-income consumers and create jobs (WorldFish Centre, 2011). Tilapia production has increased worldwide with and is now considered the 2nd most farmed species in the world and it is expected to continue to grow in tropical areas (Watanabe et al., 2002; Fitzsimmons, 2013). With the growth of industry comes the need to improve on production systems and reduce production costs as well as reduce overdependence on dwindling fish meal and fish oil sources from the wild used in feed prodcution in the industry. This will mean more research to develop better production systems, improved value chain, alternative feed ingredients and minimal environmental impact.

The small and medium scale pond sectors of the aquaculture industry in Ghana still struggles 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 using commercial feeds, feed constitute approximately 60% of the cost of production and, 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. There have been advances made in the use of agro-products as partial replacements of fish meal as the protein source in tilapia diets (Obirikorang et al., 2015).

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.

The importance of pond fertilization has been highlighted by several studies. FAO (2005) reported harvest outputs of 3 MT/ha for tilapia grown in fertilized ponds without any external feed inputs. The production of tilapia in fertilized ponds (5,135-9,000 kg/ha) was 2 to 4 times higher than in unfertilized ponds (2,240 kg/ha) (Das and Jana, 1996). Studies done by Diana et al., (1994) and Diana (2012) showed that fertilization rates of between 50-70% had fish yields comparable to ponds that received 100% of the recommended rates.

Secondly, in spite of the progress made in the development of the improved Akosombo strain of Nile tilapia in Ghana, pond farmers scattered through the regions still face a lack of access to adequate 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.

The most practical solution for pond farmers is to buy fry at 1.5 - 2 g 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 unpredictable success rates for pond-based farming of tilapia in Ghana. 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.

In this proposed study, a series of experiments and economic analyses will be performed based on lessons learned from other developing countries, including previous AquaFish funded studies, to develop a locally verified base of knowledge for pond-based tilapia producers in Ghana.

Quantified Anticipated Benefits

At the completion of this study, it is anticipated that improved knowledge of commercial feed use will translate into both increased yield and profitability through the production cycle. The Ghana Aquaculture Development Plan has a target of 100,000MT/year of domestic tilapia production by the end of 2018. Contribution of pond aquaculture to the current production of ~ 40,000MT/ year is less than 10%, in spite of high existing pond capacity and growing availability of commercial fish feeds. The US imports over 90% of seafood consumed and tilapia is among the top three fishes imported into the states (Harvey, 2016). Tilapia imports continue to grow e.g., in 2002, approximately 148 million pounds of tilapia was imported into the US compared with a little over 496 million pounds in 2015. The National Oceanic and Atmospheric administration of the Department of Commerce, in its 2016 - 2020 strategic plan for aquaculture development (NOAA, 2015), emphasizes the need to reduce reliance on fishmeal and oil as well as increase the number and types of ingredients available to feed manufacturers. This study will not only ensure that more small-scale pond farmers make profits through a more efficient use of local ingredients, commercial feeds and fertilization, but it will also raise the contribution of pond farms to national aquaculture development goals for the both Ghana and the US. It is also expected that the study will facilitate solutions to local feed problems for fish farmers through a routine use of profitable low-cost technology.

Research Design and Activity Plan

The experimental components of this study will be carried out in earthen ponds, tanks and hapas. Objectives 1, 2, 3, 4, 5, and 6 are respectively matched with Experiments E1, E2, E3, E4, E5, and E6.

Experiment E1

Hypothesis 1: Growth rate of fry/fingerling will increase with decreasing stocking density.
Hypothesis 2: Survival rate of fry/fingerling will increase with decreasing stocking density.
Hypothesis 3: FCR will decrease with decreasing stocking density.
Experiment E1 will follow a completely randomized design with two factors: 1) Density at 25, 50, 75, and 100/m^3, and 2) Time (i.e. number of days till fish are observed) at 10, 20, 30, and 40 d (Figure 1). Each experimental unit is a hapa of size 1 – 4 m^2 in a pond of 1 m water depth. All fish will be stocked at the same size of approximately 2 g and fed twice daily to satiation (or the minimum percent of body weight by size recommended by the feed manufacturer) on a 45-50% protein commercial feed. After weighing subsamples for stocking, fish will not be sampled until the predetermined day of observation, to minimize handling mortality. At the predetermined time, data will be collected and the observed experimental units will be closed. All surviving fish from each observed unit will be counted and weighed and the data used to calculate growth, survival, and feed conversion ratio (FCR) for each treatment. The experiment will be repeated with fingerlings from at least one different hatchery to provide more robust results with up to six replicates per treatment. In the event of limitation of ponds, the experiment may be carried out in batches over six months to one year.

Figure 1 – Experimental design for Experiment 1 (E1).

Density	25 fry/m^3				50 fry/m^3				75 fry/m^3				100 fry/m^3			
Days till Observed	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d

Experiment E2

Hypothesis 4: Growth rate and FCR will not differ between 30%, 28%, and 25% feeds in a fertilized pond.

Experiment E2 will be carried out in experimental 150 – 200m^2 ponds. The experiment will be run at least 2 times for a total of 18 experimental units and 6 replicates per treatment. This is a one-factor experiment at 3 levels of protein (30%, 28%, and 25% protein) following a completely randomized design. Our experience in previous experiments have indicated that the first round of an experiment may not evolve as planned and at least a second round experiment may be desired to correct errors and increase power for statistical analysis. The 30% and 28% protein feeds are available on the market in Ghana from a locally-based feed producer with whom we have worked in the past. The 25% feed will be produced under a special arrangement with the producer. Fish for this experiment will first be stocked as 2 g fry in a hapa within each pond and fed on high-protein feeds until they attain approximately 30 g before being sorted by size and sex. Males will be stocked in the experimental ponds at 2-3/m^2. Thereafter, feeding will be 2 times a day (approximately 10:00 am and 16:00 pm) starting at 4% mean body weight (mbw)/day and reducing through the growth period to about 2% mbw/day at harvest. Fish growth will be monitored biweekly to about 350 - 400 g which is projected to take 5 months per experimental run.

Experiment E3

Hypothesis 5: Growth rate will not differ between the half-ration and full-ration 30% protein feed treatments in a fertilized pond.

Hypothesis 6: FCR will be lower for the half-ration treatment compared to the full-ration treatment in a fertilized pond.

Experiment E3 will be carried out in experimental 150 – 200m^2 ponds. The experiment will be run at least 2 times for a total of 12 experimental units and 6 replicates per treatment. This is a one-factor experiment at 2 levels of feeding (30% protein; full ration and 30% protein; half ration) following a completely randomized design. Stocking, feeding and monitoring will follow protocols described for E2 with the half ration treatment receiving half of the quantities of feed as the full ration treatment.

Experiment E4

Hypothesis 7: Palatability/acceptability will not differ among the different diets.

This experiment will be run on three experimental diets formulated using locally available and affordable ingredients. Diets will have different levels of combinations of the following protein sources: palm kernel cake (PKC), Copra cake (CC), Soybean meal (SBM), and Groundnut husk (GNH) and formulated to have iso-protein and iso-energy levels that are comparable to that of the commercial diet used in E1. Rice bran and wheat bran will be the main energy sources used. This will be a 2-week study and the experimental units used will be glass tanks at KNUST lab/farm using a completely randomized design. Each treatment will have three replicates.

Experiment E5

Hypothesis 8: Survival rate will not differ among the different diets.

Hypothesis 9: Growth rate will not differ among the different diets.

Hypothesis 10: FCR will not differ among the different diets.

Experiment E5 will also be based on a completely randomized block design following E1 (Figure 1) but with the best two experimental diets from E4. The diets will also be iso-nitrogenous and iso-energetic and comparable to the commercial diet used in Experiment E1. The commercial diet used in experiment E1 will also serve as the control for this experiment. The three experimental diets plus the control diet will be fed to fish in 1 - 4 m^2 hapas mounted in ponds of water depth of at least 1m. Fish will be stocked at approximately 2g and fed twice daily to satiation on a 45-50% protein until the end of the study. The duration of this experiment will be informed by results obtained from E1 (30 – 40 days).

Experiment E6

Hypothesis 11: Phytoplankton communities will not be different among diets in E1 and E5.

Hypothesis 12: Zooplankton communities will not be different among diets in E1 and E5.

Phytoplankton and zooplankton composition and succession in ponds will also be monitored following methods described in Frimpong and Lochmann (2006) and Ludwig et al., (2010); three random samples will be taken from each experimental pond in E1 and E5 before stocking and weekly after stocking.

Location

Experiments will be carried out in aquarium tanks and ponds at the Fish Farm at Kwame Nkrumah University of Science and Technology, Kumasi. Other partners' (e.g., Pilot Aquaculture Center of the Fisheries Commission, PAC) facilities will be used as needed.

Methods

All experimental ponds will be fertilized with the same AquaFish recommended protocol. After draining and drying ponds for 2 weeks to 1 month (depending on weather), agricultural limestone ($CaCO_3$) will be applied to the pond bottom and the slopes of the dykes at a rate of 100 g/m^2. Seven days later, the ponds will be filled to 1 m depth and stocked one week after fertilization begins. Fertilization will be applied once a week at 4 kg Nitrogen and 1 kg Phosphorus/ha/day, using urea and diammonium phosphate. The 1 m pond depth will be maintained with periodic topping up of water as needed. Routine water quality measurements will also apply to all ponds on a weekly basis using Hanna multiparameter meter HI 9828. Variables to be monitored include temperature, dissolved oxygen and pH. In addition, Secchi disk depths will be taken once a week to monitor the effectiveness of the fertilization. Experimental data on growth, survival and FCR will be analyzed for each experiment using Analysis of Variance (ANOVA) techniques. Repeated measures ANOVA will be run on data from the control and the experimental diets, after which a post-hoc analysis will be run to test for differences among treatments means.

Diet palatability/acceptability of experiment diets will be assessed subjectively by directly observing fish feeding responses/behavior and also by quantifying feed intake in tanks. Temperature, pH and dissolved oxygen will be monitored daily for two weeks. Phytoplankton samples (100 mL) will be preserved by adding 0.5 mL formalin and 1 mL Lugol's solution. Phytoplankton in the samples will be quantified and identified to genera level (Ludwig et al., 2010). For the zooplankton, samples will be preserved in 70% isopropyl (Frimpong and Lochmann, 2006; Ludwig et al., 2010). Zooplankton in the samples will be identified to the three major groups – rotifers, copepods and cladocerans.

The experimental data will provide input to economic analysis of relative profitability of the feeding strategies. Standard methods for profitability analysis, including enterprise budgets and partial budgets will follow Engle and Neira (2005). Feed prices for estimating the cost of production will be 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 will also be obtained from the market. Based on available data, the 25% protein feed, for example, is priced about 15% less/kg compared to the 30% protein feed. It is straightforward 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. All data for profitability analysis will be organized in Microsoft Excel and Monte Carlo analysis using @Risk software embedded in Excel will ensure that various forms of uncertainty in prices and the experimental results will be accounted for.

<u>Trainings and Deliverables</u>

There will be 1 training/workshop for 100 tilapia farmers (hatchery operators, nursery operators and potential operators and fish farmers, and aquaculture students) on optimal stocking densities and optimal protein levels and feeding strategies. This will also serve as a means of disseminating results from the project. Deliverables will be:

1. A training/workshop will be conducted for at least 100 tilapia farmers in optimal production techniques;
2. Final Report;

3. At least four peer reviewed publications: (best fry stocking densities for optimum growth, performance of the new fingerling feed, best protein levels for optimal returns and plankton composition and succession);
4. Four MSc. and one BSc. students trained in tilapia production system design and management.

Schedule

ACTIVITIES	2016/2017											
	M	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**
Inception meeting	■											
Pond preparation for round 1 experiments		■	■									
Stocking of fry and fingerlings			■									
Preparation of experimental diets	■	■	■									
Acceptability/Pal. trial- experimental diet			■	■	■							
Weekly fertilization & Water quality monitoring		■	■	■	■	■	■	■	■			
Biweekly fish growth monitoring			■	■	■	■	■	■	■			
Plankton sampling and identification			■	■	■	■	■	■	■	■	■	■
Fish harvesting								■	■			
Profitability Analysis								■	■	■		
Data analysis								■	■	■	■	■
Pond preparation for round 2 of experiments												■
	2017/2018											
	M	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**
Pond preparation for round 2 of experiments	■											
Stocking of fry and fingerlings		■										
Weekly fertilization & Water quality monitoring	■	■	■	■	■	■	■					
Biweekly fish growth monitoring		■	■	■	■	■	■					
Plankton sampling and identification	■	■	■	■	■	■	■	■	■	■		
Fish harvesting						■	■					
Profitability Analysis					■	■						
Data analysis & report writing						■	■	■	■			
FINAL REPORT WRITING									■	■	■	■

A COMPARISON OF MONOCULTURE AND POLYCULTURE OF TILAPIA WITH CARPS FOR POND PRODUCTION SYSTEMS IN NEPAL

ASIA PROJECT: NEPAL
US Project PI: James Diana, University of Michigan
HC Project PI: Madhav Shrestha, Agriculture and Forestry University

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

Collaborating Institutions and Lead Investigators

University of Michigan (USA) — James Diana
Agriculture and Forestry University (Nepal) — Narayan Pandit, RN Mishra, SK Wagle, Madhav Shrestha

Objectives

1. To compare monoculture and polyculture of Nile tilapia with carps on-station and on-farm for optimal production.
2. To evaluate the value of each management manipulation by comparing enterprise budgets for each treatment and pond system.

Significance

Total fish production in Nepal was 54,357 metric tons in 2012, with about 60% originating from aquaculture. Pond culture is the most popular method of aquaculture, but annual pond yield averages only 3.83 t/ha (DoFD, 2012). Carps are popular warmwater 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*) and tilapia (Pandit et al., 2004), and recently additional polyculture experiments 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). Experiments indicated that sahar can sometimes control tilapia fry production, but often result in overpopulation of tilapia even when sahar are present (Paudel et al., 2007; Rai et al., 2007; Yadav et al., 2007; Shrestha et al., 2011). Growth of sahar was higher in tropical and subtropical ponds than in cages reared in Pokhara lakes, as well as suspended cages in ponds (Bista et al., 2001, 2007; Shrestha et al., 2005, 2007). Sahar has been overfished in rivers and lakes, which has resulted in declining populations (Rajbanshi, 2001; Joshi et al., 2002; Rai et al., 2007).

Semi-intensive carp polyculture is an established system in the 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 will 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.

We recently completed 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. Overall production was still relatively low, about four tons per ha annually. This overall production is lower than monosex tilapia culture in ponds in Thailand, where we have achieved 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. Additionally, sahar are an endangered species (IUCN, 2016), so any success in rearing them will either relieve pressure on wild populations as a food source or will be used to supplement wild populations by stocking, again worthy goals to improve 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 proposal will focus on this 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 (GoN 2000, NARC 2010). Again, this project is in complete alignment with these goals.

This study is 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 will conduct 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.

Quantified Anticipated Benefits

The results of this study will provide two additional species in the aquaculture system of Nepal, which should increase production and income. It will add high-valued fish to the culture system and supplement income. As carp polyculture is established, the increasing 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. Polyculture will also help in production of sahar, which could be stocked in natural waters to reverse population declines. It will benefit fish culture in southern Asia and other countries where carp culture is popular. The immediate impact will be measured by the increased production and economic returns in on-station and on-farm trials for the different polyculture systems. We expect to improve yield and economic returns for aquaculture systems by at least 30% over traditional carp culture and train at least 5 farmers in the new production system. We will document these benefits through on-station trials and subsequent surveys of farmers who attend our training sessions.

Research Design and Activity Plan

Location

Aquaculture Farm, AFU, Rampur, and private farm ponds in fish production pockets of the Chitwan district.

Methods

Null hypothesis: Monoculture of monosex tilapia produces significantly higher yields and profits than polyculture under current conditions.

1.1 Test species: carps (common, silver, bighead, grass, rohu, mrigal and catla), Nile tilapia, and sahar

1.2 Stocking:
carps (5-10 g) at 10,000/ha
Nile tilapia (5-10 g) at 3,000/ha in polyculture, 10,000 or 20,000/ha in monoculture
sahar (5-10 g) at 1,000/ha

1.3 Nutrient input: daily feeding with locally made feed (20% CP) composed of 1:1 mustard oil cake (28% CP) and rice bran (12% CP) at 2% of total biomass per day. Ponds fertilized biweekly at 0.4 g N and 0.1 g P $m^{-2}day^{-1}$ with di-ammonium phosphate (DAP) **(**18% N and 46% P_2O_5), **urea** (46% N) and farm yard manure (FYM)**.** DAP and urea input at 700 and 940 g, respectively, and FYM at 60 kg for a 200 m^2 pond.

1.4 Water management: maintain at 1 m deep.

2. Treatments:

2.1 Existing carp polyculture (10,000/ha) + mixed-sex tilapia (3,000/ha) + sahar (1,000/ha)

2.2 Existing carp polyculture and monosex tilapia at 3,000/ha.

2.3 Monosex tilapia at 10,000/ha with fertilization only

2.4 Monosex tilapia at 20,000/ha with fertilization and feeding

3. Each treatment will be tested in triplicate ponds at the Aquaculture Farm, AFU, Rampur. Additionally, each treatment will be tested in private farm ponds in Chitwan with at least three replications of each treatment.

4. Sampling Schedule: Water quality will be measured monthly in the on-station work and at initiation and harvest times in each on-farm pond using CRSP protocols. Fish growth and yield will be measured monthly from stocking to harvest in on-station trials but only at stocking and harvest in on-farm trials. Partial enterprise budgets will be estimated for overall production results of each treatment in both pond systems.

5. Statistical Design and Statistical Analysis:
The trial will be conducted in a completely randomized design, and data will be analyzed using one-way ANOVA within each research site. Evaluation of differences in values for a given treatment type between on-farm and on-station trials will be done by t-test.

Trainings and Deliverables

Training:
30+ farmers will learn new culture systems in on-farm trials
Workshop to train on-farm plus other farmers in system technology
At least 5 graduate and undergraduate students will receive research training by working on the trials

Deliverables:
Testing results of 2 new polycultures and 1 monoculture system
Improve yield and economic return by 30% for farmers, documented by the outcome of our on-station and on-farm trials.
One fact sheet on use of tilapia in aquaculture in Nepal
One final report and hopefully one research publication to be completed after the grant period.

Extend results to 30+ farmers through the demonstration project and a workshop.
Train at least 5 graduate and undergraduate students on aquaculture research.
Final extension to other farmers through a fact sheet.

Schedule

Stocking of on-station and on-farm ponds in April 2017. Harvest of ponds in November 2017. Final report no later than February 2018. Workshop in December 2017.

DEVELOPING NEW SYSTEMS FOR PERIPHYTON ENHANCEMENT IN FARMERS' PONDS

ASIA PROJECT: NEPAL
US Project PI: James S. Diana, University of Michigan
HC Project PI: Madhav K. Shrestha, Agriculture and Forestry University

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

Collaborating Institutions and Lead Investigators

University of Michigan (USA)	James S. Diana
Agriculture and Forestry University (Nepal)	Sunila Rai
	Kamala Gharti
	Madhav K. Shrestha

Objectives

1. To identify other possible methods besides bamboo rafts to enhance periphyton growth in ponds while not interfering in harvest systems.
2. To field test the most promising of these methods for periphyton enhancement in ponds in additional on-farm trials.

Significance

The government of Nepal (GoN) has recognized that chronic malnutrition is a major problem in the country. The most common forms of malnutrition include undernutrition (insufficient energy) and deficiencies of vitamins and minerals, particularly vitamin A, iodine, and iron. About 41% of children less than 5 years of age are stunted (UNICEF, 2012a) 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 rural poor 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 et al., 2008). As a result, most of the ponds developed for household aquaculture are managed by women.

Since 2008, the Institute of Agriculture and Animal Science (now the AFU) has promoted an innovative and environmentally sustainable fish production system of "Carp-SIS polyculture" to improve nutrition of poor women and children in Terai (Rai, 2012, 2013). The approach includes increased intake of small indigenous fish species (SIS) to improve health and nutrition of women and children. SIS are self-recruiting in aquaculture ponds, after initial stocking or colonization from natural waters, and can be harvested weekly and biweekly, favoring regular household consumption. A carp-SIS polyculture system also provides additional income through the sale of surplus fish. Addition of SIS to the carp polyculture system raised fish production above the national average, doubled consumption rate of household members, and provided Rs. 3,025 income per household in 270 days, which helped families become economically empowered (Rai, 2012).

We have done considerable research to help improve the carp polyculture system in Nepal by introducing new species (especially SIS, tilapia, and sahar) and by enhancing pond production by providing 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%) 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 meetings with farmers and testing of alternative periphyton enhancing substrates, 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. Since these issues were identified by farmers, we intend to hold two workshops to meet with farmers and identify their best ideas to develop periphyton substrates that will minimize disturbance to their operations. We then plan to field test the various methods identified in farm ponds.

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.

The purpose of this study is to assess 2-3 alternative periphyton enhancement methods identified by farmers in farm ponds with polyculture of carps and SIS in two locations of Nepal.

Quantified Anticipated Benefits

We anticipate that our workshops will identify new methods for periphyton to be enhanced in ponds, and about 40 farmers will adopt these systems after testing in on-farm trials. We will also hold a workshop for government personnel and farmers to extend the results of these experiments to these user groups.

Extension of periphyton enhancement to 20 farms through on-farm trials. Education of another 40 women will be done with a workshop to non-adopting farmers and 20 extension personnel during a workshop for government personnel, extending the results of our periphyton work. At least 5 graduate or undergraduate students will be involved in some aspect of this research, also enhancing their educational and research experience.

Research Design and Activity Plan

This experiment will evaluate increased yield of carps and SIS as a result of enhancing periphyton production, and will also determine improvements in water quality as a result of periphyton treatments.

Location

On-farm verification of the best system will be tested in farmers' ponds in two districts; at Majhui, Chitwan and Kawasoti, Nawalparasi.

Methods

Null hypothesis: There are no differences in growth, production, gross profit margin, and partial harvest among different polyculture systems (control, different periphyton substrates.

1. Workshops: Two workshops will be held: one in Majhui and one in Kawasoti. At the workshops, farmers from the region that have been involved in previous periphyton studies will be asked to propose better alternatives for periphyton substrates in ponds. These alternatives must be environmentally responsible, including the materials used for colonization. The best alternatives will then be determined by voting of all attendees for all alternative designs suggested. Probably 2-3 alternatives will be selected for future testing, but the number are currently uncertain.

2. Experiment:

2.1 Culture period: 6 months for on-farm trials, SIS monitored for 12 months.

2.2 Test species: carps (common, silver, bighead/catla, grass, rohu, mrigal) and SIS (dedhuwa and pothi)

2.3 <u>Stocking and Treatments</u>
Stocking size: carps (5-10 g), SIS (2-5 g)
2-3 periphyton enhancements will be tested in multiple ponds
Additionally, a control with reduced feeding and no periphyton enhancement will be tested.
Besides the substrate systems tested, the inputs will be:
(1) Carp polyculture (15,000/ha) + SIS (50,000/ha) with 50% feeding (control)
(2) Control + substrate (covering 2% of pond surface area) with 50% feeding. There will be multiple versions of these treatments, including various substrate enhancements identified in the workshops.
Overall production levels, fish size, and SIS yield will be evaluated for each farm by record keeping during harvest. Growth and production will only be assessed over the whole grow out season. Weight will be measured on 20 individuals of each species at stocking and harvest. Total harvest biomass will be measured for each species.

2.4 Nutrient input: feeding (for reduced rations) will be done 6 days per week with dough of rice bran and mustard oil cake at 1.5% BW for most carps and grass to grass carp at 50% BW. Carp and SIS will be fed with freshly made dough of mustard oil cake and rice bran (1:1). Grass carp will be fed daily with locally available grass at 50% body weight. Fertilization will be done biweekly at 0.4 g N and 0.1 g P $m^{-2}day^{-1}$ with di-ammonium phosphate (DAP) (18% N and 46% P_2O_5), urea (46% N) and farm yard manure (FYM). DAP and urea input at 700 and 940 g, respectively, and FYM at 60 kg for a 200 m^2 pond.

2.5 Water management: maintain at 1 m deep.

2.6 Water quality will be measured at stocking, mid-point, and harvest by project personnel. Parameters will include Dissolved Oxygen, SDD, and temperature will be measured as close to dawn as possible on each date. Periphyton colonizing substrates will be identified to species by microscopy and quantified for relative abundance at the beginning, middle, and end of the experiment in each pond.

2.7 Farm visits will be made to each location at stocking, mid-point, and harvest to evaluate record keeping as well as to make measurements of fish and water quality variables.

2.8 <u>Statistical design and statistical analysis</u>:
Statistical design: Completely randomized design (CRD)
Statistical analysis: Multiple ANOVA

<u>Trainings and Deliverables</u>

Training: Twenty farmers will learn periphyton enhancement techniques through on-farm trials.
Forty women will learn about this technology through a workshop.
Twenty extension personnel will also be trained on the technology through a workshop.
At least five graduate or undergraduate students will be trained on research methods by involvement in these trials.

Deliverables: Two workshops to determine alternative periphyton technologies.
One workshop on periphyton technology results.

One fact sheet describing this technology.
One final report and hopefully one research publication after the end of this grant period.

Schedule
Periphyton workshops: 1 October 2016 through 1 January 2017. On-farm trials: 1 May 2017 through 1 December 2017. Final report will be completed no later than 28 February 2018.

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

AFRICA PROJECT: KENYA & UGANDA
US Project PI: Joseph J. Molnar, Auburn University
HC Project PI: John Walakira, National Fisheries Resources Research Institute

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

Collaborating Institutions and Lead Investigators

Auburn University (USA)	Claude Boyd Joseph J. Molnar Shamim Naigaga (PhD Student)
National Fisheries Resources Research Institute (Uganda)	Gertrude Atukunda Moureen Matuha John Walakira

Objectives

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.

Significance

Previous AquaFish research compared the results of several commercially available water quality test kits compared to standard methods. The results suggest that water analysis kits can be used to make aquaculture decisions that are as good as those drawn from standard methods, except when measuring nitrate concentrations (Naigaga, 2015).

The present study builds on the water quality testing results to profile aquaculture waters in Uganda. The study will obtain information on water quality in reservoirs, lakes, and ponds, ponds soil characteristics, aquaculture management, climatic conditions and pond hydrology, and quality of liming materials in Uganda. These data will be assessed and recommendations on water, water quality, and pond soil management in Ugandan aquaculture will be developed. NaFIRRI researchers will collaborate and support the water sampling collection.

Quantified Anticipated Benefits

- Manual on pond soil and water best management practices.
- Three presentations to fish farmer cooperatives will address technical needs and issues, as well as specific gender-related concerns in the operation of farmer associations.
- Climate change impacts on aquaculture and possible ways for mitigation.
- Increases awareness on the water and soil quality for Ugandan aquaculture among farmers, extension workers, Government and institutions hence better networking.

Research Design and Activity Plan

Location

Aquaculture Research and Development Center-Kajjansi.[1]

Methods

Water samples will be collected from at least 50 ponds across the Uganda aquaculture area, and from reservoirs and lakes in Uganda that have appreciable amounts of aquaculture. The pH, temperature, and dissolved oxygen concentration will be measured at time of collection. Water samples of 1 L, each stored in plastic bottles, will be 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 will be collected from five locations in each pond, samples from each pond will be combined into a composite sample, dried at 60 ^{0}C in an oven, and shipped to Auburn University. The samples will be analyzed for pH, organic carbon, cation exchange capacity, phosphorus, nitrogen, major ions, trace metals, free carbonate, and particle size.

While in Uganda, we will obtain weather records from at least one location, but preferably from several locations. The records should contain monthly average temperatures, maximum and minimum temperatures, rainfall, and if possible, pan evaporation. We also will measure the water loss rate from five to ten representative ponds over a period of two to four days without rainfall with aid of a stilling well and hook gauge (Yoo and Boyd, 1994).

We will collect samples of liming materials (200 g each) used in Uganda. These samples should come from several sources. They will be analyzed for neutralizing value and particle size at Auburn University.

We will collect information on typical production levels in ponds, amounts of fertilizers, liming materials, feeds, and other inputs. The use of aeration in ponds will be noted. If aerators are used, information on type, size, and typical operating schedule of aerators will be obtained. The data will be used to conduct an assessment of the suitability of water and soil quality for aquaculture in Uganda. This assessment will include the following:

- General water quality conditions – including one or more maps showing variation in water quality across regions. The possible influence of water quality in lakes, ponds, and reservoirs will be considered.
- Generalized water budgets for rain-fed ponds will be estimated to ascertain likely variation in water levels. Estimates of water inputs necessary for ponds filled from external sources also will be made.
- The need for liming will be evaluated and the quality of local liming materials will be determined.
- An assessment of other water quality amendments that might be required in ponds will be made.

---

[1] Additional resources can be found at:
http://aquafishcrsp.oregonstate.edu/Documents/Uploads/FileManager/Pond%20Dynamics:Aquaculture%20Collaborative%20Research%20Data%20Reports_Volume%201.pdf
http://aquafishcrsp.oregonstate.edu/page/globalexp
http://aquafishcrsp.oregonstate.edu/globalexp/

- Climatic variation over the country will be assessed for possible effects on aquaculture.
- The assessment results will be used to prepare recommendations for water, water quality, and pond bottom soil management in Ugandan aquaculture.

Trainings and Deliverables

Item	Mechanism (e.g. podcast, reports, factsheets).
Information on water and soil quality	Water and soil quality manual
Awareness raising and networking	Conference
Information on climate change effects on aquaculture	Aquaculture and climate change manual

Schedule

Task	10/16	1/17	3/17	6/17	12/17	2/17
Document development	x	x				
Collecting data		x	x	x	x	x
Collecting and analyzing data	x	x	x	x	x	x
Dissertation writing	x	x	x	x	x	x

TOPIC AREA

SUSTAINABLE FEED TECHNOLOGY AND NUTRIENT INPUT SYSTEMS

❖

PELLET FEED IMPROVEMENTS THROUGH VITAMIN C SUPPLEMENTATION FOR SNAKEHEAD CULTURE

ASIA PROJECT: CAMBODIA & VIETNAM
US Project PI: Robert Pomeroy, University of Connecticut
HC Project PI: So Nam, Inland Fisheries Research and Development Institute

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

Collaborating Institutions and Lead Investigators

University of Rhode Island (USA)	David Bengtson
	Marta Gomez-Chiarri
Can Tho University (Vietnam)	Tran Thi Thanh Hien
	Tran Ngoc Hai
Inland Fisheries Research and Development Institute (Cambodia)	Chheng Phen

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 (Vietnam); and (ii) to evaluate cost-effectiveness of pellet diets with optimal vitamin C for hapa growout (Cambodia).

Significance

Aquaculture of freshwater carnivorous and omnivorous fish species in Cambodia and Vietnam has been highly dependent on inland fisheries of low value fish for sourcing key dietary nutrient inputs. Adequate pelleted diets, with minimal content of fish meal (FM), are needed to overcome the use of small-size fish (SSF) harvested from the Mekong for aquaculture. From 2007-2015 researchers at Can Tho University (CTU) in Vietnam, working with US collaborators at the University of Rhode Island (URI) developed a formulated feed that reduces the use of SSF and FM content without decreasing growth performance and marketability. Results of the research were disseminated directly to feed manufacturers and more than ten aquaculture fish feed manufacturers in the Mekong Delta now make pelleted diets containing a mixture of FM and soybean meal (SBM). In 2015, more than 90% of snakehead farmers (who produce 99% of the total production of snakehead) in 13 provinces 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. In An Giang, Dong Thap and Tra Vinh provinces, mainly snakehead culture provinces, about 2500 farmers now use pelleted feed. However, in the latest phase of this project, in our commercial-scale farm trial in An Giang province, about 20% of fish that were fed this diet (vs. an SSF diet) developed vertebral column abnormalities. 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 (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. 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 et al., 2012; Duc et al., 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 *Channa micropeltes* (or any other member of the family Channidae for that matter), the requirement for most fish species are considered to be 50 mg/kg (NRC, 1993). One study of the related *Channa punctata* indicated that 2 g/kg of AA provided greater resistance to toxicity of the pesticide endosulfan than did 1 g/kg, i.e., 2000 mg/kg vs. 1000 mg/kg (Sarma et al., 2009). The amount of AA that we used in our previous research in laboratory tanks and farm trials (80-150 mg/kg) (Hien et al., 2015; Hien et al., submitted *a*) appeared to us sufficient at the time. We saw a few abnormal fish in the laboratory experiment or pond trials at CTU. In the 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. 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 we anticipated.

We have concentrated our research, development and outreach efforts in Vietnam due to the ban on snakehead culture in Cambodia. 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 our research 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 can be lifted. With the lifting of the ban on snakehead aquaculture in April 2016, the research will focus on snakehead culture technology (breeding, hatchery-rearing, and pond culture) being transferred from Vietnam to Cambodia scientists, specifically to the Fisheries and Aquaculture Research and Development Center (FARDeC) facility at Prey Veng. The next stage of the technology transfer should cover the dietary aspects of snakehead culture (fish nutrition and feed formulation). We propose that Dr. Hien of CTU will lead the work to provide training on fish nutrition, feed formulation and feeding strategies to IFReDI staff at CTU, including a trip to snakehead feed manufacturers in Vietnam and training in equipment use and conducting fish nutrition studies. This will be followed by several trips by CTU personnel to Cambodia for testing improved snakehead feed and installation of software for feed formulation at FARDeC.

Quantified Anticipated Benefits

The results of this study will further define optimal pelleted diets for snakehead in Vietnam and eliminate the hunchback abnormality problem and also improve fish health seen on some farms there. Perhaps more importantly, it will provide further demonstration to the Cambodian government that snakehead culture based on pelleted feed should be allowed in Cambodia. Finally, it will train IFReDI personnel to be able to conduct their own future studies in fish nutrition and feeding for snakehead and other species of interest.

These experiments will also allow the U.S. Participant in this investigation, Dr. Bengtson, to continue his studies of plant protein replacements for fish meal from temperate to tropical species.

The experiments will result in undergraduate and graduate thesis research at CTU, as well as training of IFReDI personnel, providing further capacity building in trained graduates who will work in the aquaculture industry in the Lower Mekong Basin.

On a broader scale, use of plant products to replace fish products in aquaculture diets is a major area of research worldwide. This project will add to the knowledge base for the formulation of diets that will make aquaculture more sustainable globally, as have our previous publications.

Finally, further adoption of soybean-based diets by the snakehead industry will provide increased markets for operators of feed-mills and potentially for the U.S. soybean industry.

Research Design and Activity Plan

Location

- Formulation of diets will be done through collaboration between CTU and URI based on information about chemical composition of ingredients. Manufacture of the diets will be done at CTU, which has a small fish-feed mill (for floating feed, 200kg/hour), as will analysis of diet composition: protein, lipid, mineral, fiber, and energy.
- The laboratory feeding trial will be conducted in a wet lab at CTU.
- The post-feeding trial bacterial challenge experiment will also be done in a wet lab at CTU.
- A pond trial will be conducted in experimental ponds with hapas at CTU by CTU researchers.
- Training of IFReDI researchers will take place at both CTU and FARDeC.
- Installation of software for feed formulation will take place at IFReDI.
- Based on the CTU pond trial results, a confirmatory pond trial will take place at FARDeC.

Methods

This study will comprise interrelated parts:

(i) Effects of variation in dietary vitamin C (AA) in snakehead.

Null hypothesis: AA level does not lead to significant differences in performance of snakehead, as measured by survival, growth, feed conversion ratio (FCR), protein efficiency ratio (PER), certain blood parameters (see below).

Based on the several benefits attributed to AA in other studies of fish nutrition, and the basal diet from our previous work with SBM replacement of FM in diets for snakehead fish, we propose to test the null hypothesis that AA level does not lead to differences in performance of snakehead. Although our previous work with diets based on soy protein concentrate (SPC) supplemented with mannan oligosaccharides (Hien et al., submitted *b*) was very promising, availability of SPC in Southeast Asia is problematical, so we choose to work with the more available SBM. We will conduct a feeding trial in the CTU laboratory, followed by a bacterial challenge, as outlined below. The lowest supplemented AA value is in the mid-range of what we have been using, the highest supplemented levels are those used by Sarma et al., (2009), and we have included two intermediate values in between. The feed will be analyzed to verify AA according to Nelis et al., (1997).

1. SBM diet + 0 mg AA/kg feed
2. SBM diet + 125 mg AA/kg feed
3. SBM diet + 250 mg AA/kg feed
4. SBM diet + 500 mg AA/kg feed
5. SBM diet + 1000 mg AA/kg feed
6. SBM diet + 2000 mg AA/kg feed

The laboratory experiment will be conducted in a manner similar to those we conducted previously (Hien et al., 2015). Experimental units are 500-L tanks. In this trial we will use seven replicate tanks per treatment for greater statistical power, especially for the subsequent bacterial challenge. The stocking density will be 80 fish/tank. At the beginning of the experiment, fish (initial weight about 4-5 g) will be weighed. Fish will be fed to satiation twice a day (0800 and 1600 hrs) and the amount of feed consumed by the fish in each tank will be recorded daily by removing and weighing (dry weight) excess feed to ascertain intake. Amounts of feed provided per replicate will be recorded so that feed conversion ratio (FCR) and protein efficiency ratio (PER) can be calculated at the end of the experiment. The water will be maintained at 28±2°C. Water quality (temperature, pH, DO) will be measured by meters twice daily (0700 and 1500 hrs) and ammonium, nitrate and nitrite will be measured weekly by test kit. Any dead fish will be recorded and removed daily. The experiment will last eight weeks, at the end of which fish will be measured, weighed and then used in the bacterial challenge experiment. Blood samples of a subset of experimental fish will be taken at the end of experiment and examined for white blood cell count, red blood cell count, lysozyme activity and glucose level, using methods that we have used in our research during the last two years (Hien et al., submitted b). Any skeletal disorders will be documented via photographs and X-rays. Data from a tank are pooled (i.e., no pseudoreplication) and only one number representing average growth per fish (specific growth rate, SGR) is used per replicate. Based on the cost of feed ingredients used for each diet, feed cost per kg of fish produced will be calculated for each treatment. Data analysis is by one-way analysis of variance (ANOVA), following arc-sine square-root transformation of the proportionate data to insure normality. Tukey's HSD test is used to determine specific differences among means if the ANOVA indicates that significant differences are present.

(ii) Bacterial challenge experiment

Null hypothesis: AA levels in the feeding trial above do not lead to significantly different survival in a post-trial bacterial challenge.

The bacterial challenge experiment will be conducted at the end of the feeding trial. The six treatments in the feeding trial will be subdivided, such that fish from five tanks per treatment will be 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 (Duc et al.., 2013) and fish from two tanks per treatment will be IP injected with 0.1 mL of physiological saline (0.85%) as control (Ward et al., 2016) (tanks will be randomly selected prior to the feeding trial to avoid bias). This bacterial challenge experiment will last 2 weeks, as in our previous work (Hien et al., submitted *b*). During the 14 days post-inoculation, fish will continue to be fed their respective diets, and activity and cumulative mortality will be noted daily. For moribund fish, clinical signs will be observed by gross inspection, and lesions will be sampled directly for bacteria. Re-isolation and re-identification of bacteria will be carried out according to methods of Barrow and Feltham (1993) and PCR method will be used to speciate the re-identified bacterial strains. The cumulative mortality will be recorded daily.

1. SBM diet + 0 mg AA/kg feed (saline 0.85% injection), control (2 reps)
2. SBM diet + 0 mg AA/kg feed (*Aeromonas hydrophila* injection) (5 reps)
3. SBM diet + 125 mg AA/kg feed (saline 0.85% injection), control (2 reps)
4. SBM diet + 125 mg AA/kg feed (*Aeromonas hydrophila* injection) (5 reps)
5. SBM diet + 250 mg AA/kg feed (saline 0.85% injection), control (2 reps)
6. SBM diet + 250 mg AA/kg feed (*Aeromonas hydrophila* injection) (5 reps)
7. SBM diet + 500 mg AA/kg feed (saline 0.85% injection), control (2 reps)
8. SBM diet + 500 mg AA/kg feed (*Aeromonas hydrophila* injection) (5 reps)
9. SBM diet + 1000 mg AA/kg feed (saline 0.85% injection), control (2 reps)
10. SBM diet + 1000 mg AA/kg feed (*Aeromonas hydrophila* injection) (5 reps)
11. SBM diet + 2000 mg AA/kg feed (saline 0.85% injection), control (2 reps)
12. SBM diet + 2000 mg AA/kg feed (*Aeromonas hydrophila* injection) (5 reps)

Data will be analyzed by the Kaplan-Meier log-rank survival test, with pairwise Holm-Sidak multiple comparison procedure where appropriate (Ward et al., 2016).

(iii) Hapa trial based on SBM diet with optimal vitamin C (AA) level in snakehead

Null hypothesis: 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.

Based on the optimal vitamin C (AA) requirement in snakehead from the results of the first experiment, we will test the effects of vitamin C (AA) in hapas to simulate farm conditions, as follows:

1. Commercial feed
2. Commercial feed + hand mixed optimal AA concentration requirement
3. Commercial feed + hand-mixed 1.5X optimal AA concentration requirement
4. Commercial feed + hand mixed 2X optimal AA concentration requirement
5. SBM diet without vitamin C (AA)
6. SBM diet + optimal AA concentration requirement
7. SBM diet + 1.5X optimal AA concentration requirement
8. SBM diet + 2X optimal AA concentration requirement

The experiment will be conducted in eight experimental ponds with three replicate hapas each. Stocking density will be 100 fish/m^2 and culture period will be 5-7 month until market size is attained. We will collect data on water quality parameters daily (as described above) and fish survival and growth monthly. We will also calculate feed cost of production per kg of fish (as described above). Any skeletal disorders will be documented at the end of the experiment by photographs and X-rays. Data on fish survival and growth, FCR and PER will be statistically analyzed by two-way analysis of variance.

<u>Trainings and Deliverables</u>

Publish one peer-reviewed journal article in English and one peer-reviewed journal article in Vietnamese, plus one fact sheet.

Training of IFReDI researchers by CTU researchers will take place at both CTU and FARDeC

CTU investigators will conduct a series of short-term training programs for IFReDI researchers in fish feeding, nutrition and processing

Short-term training will include: (1) fish nutrition and data analysis (theory and practical work); (2) snakehead feed formulation and manufacture (theory and practical work); (3) feeding strategy; and (4) improved snakehead processing technology.

CTU will help install feed formulation software at IFReDI

Following the training of IFReDI personnel at CTU, and based on the products available at the time, IFReDI and CTU personnel will decide on the optimal feed formulation software to be installed at IFReDI for future studies on aquaculture nutrition.

CTU will assist with a pond (hapa) feeding trial based on SBM diet at FARDeC

Based on the results of the pond trial at CTU, CTU researchers will work with IFReDI scientists to conduct a pond (hapa) feeding trial at FARDeC to confirm the CTU results using Cambodian snakehead. This trial will only use two ponds, one for the commercial feed without added AA and the other for the best-performing diet treatment from among the other seven treatments in the CTU pond trial with fish in three hapas in each pond. IFReDI personnel will assist with diet manufacture at CTU so that they learn how to conduct nutrition and feeding experiments from diet formulation and manufacture through conduct of the feeding trial and data analysis. Methods for this trial will follow those used at CTU. The null hypothesis will again be that there is no difference between the treatments. Statistical analysis will be by t-test. Financial data will be recorded from this trial (e.g., cost of fingerlings, cost of feed, hours of labor required, sale price of product, etc.), so that an economic analysis can be made of the profitability of snakehead aquaculture in Cambodia.

Long-term training of students at CTU

Two master's (MSc) students at CTU will conduct their thesis research as part of research items (i) – (iii) above.

Schedule

The duration of implementation of this proposed investigation will be 24 months, starting from 1 March 2016 to 28 February 2018.

Year 1 (March 2016 – February 2017):

- Dietary Vitamin C ascorbic acid (AA) feeding trial
- Bacterial challenge experiment
- Sample analysis and report writing
- CTU researchers will train IFReDI researchers in feeding and nutrition studies at CTU

Year 2 (March 2017 – February 2018):

- Hapa feeding trial at CTU
- Hapa feeding trial at FARDeC
- CTU will help install feed formulation software at IFReDI
- Sample analysis and report writing

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

ASIA PROJECT: BANGLADESH
US Project PI: Russell Borski, North Carolina State University
HC Project PI: Shahroz Mahean Haque, Bangladesh Agricultural University

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

Collaborating Institutions and Lead Investigators

North Carolina State University	Russell Borski
	Peter Ferket
Bangladesh Agricultural University	Mst. Kaniz Fatema
	Shahroz Mahean Haque

Objectives

1. Evaluate the effectiveness of nutritional conditioning on tilapia feed efficiency and production.
2. Identify key factors (gene networks) associated with improved nutrient utilization 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 in fish.

Significance

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 (Angel and Ashwell, 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 (Angel and Ashwell, 2010).

A better understanding of how finfish acquire and utilize nutrient inputs is requisite for future improvements in aquaculture production efficiency because 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 vertebrates, including fish. A few studies have looked at energy uptake and utilization following conditioning in rainbow trout (*Oncorhynchus mykiss*) and the 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 digestion (Geurden et al., 2007). 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 will evaluate if nutritional conditioning can be applied to tilapia, and assess if reductions in the amount of protein in the diet of larval tilapia can 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 will evaluate the suite of genes in the intestine that are differentially expressed in fish that are fed a protein deficient diet early in larval development versus those who received a diet containing traditional levels of protein. This analysis will further our understanding of how protein uptake efficiency 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 aquacultured finfish, new research has shown that probiotic maintenance of beneficial gut flora can promote growth, greater nutrient availability, and better 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 will further build 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. Together, the identification of beneficial microbes that improve protein uptake and nutrient utilization may benefit current research into the application of probiotic supplements in feed for further enhancement of feed efficiency in fish.

This investigation will target 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. We will determine the proper length of time for nutritional conditioning of Nile tilapia larvae that will lead to enhanced feed efficiency with minimal impact on growth and survival of the fish. If successful, this refined strategy will be tested in tilapia pond culture in Bangladesh using the alternate day feeding strategy, an approach that could provide substantial costs savings for tilapia farmers in Bangladesh while also mitigating negative environmental impacts associated with excessive nutrient loading.

Quantified Anticipated Benefits

1. A new method of nutritional conditioning in rearing post yolk-sac fry will be developed that improves nutrient uptake and utilization and production efficiency of tilapia in culture.
2. The technology has potential to increase household income of farmers in Bangladesh, thereby contributing to greater food security. The work could also be applied to improving returns for farmers in the US who predominantly culture tilapia in recirculating aquaculture systems.

3. Improvement of environmental water quality and fish stock health through enhanced nutrient utilization in tilapia pond culture could be realized through use of nutritional conditioning and reduced nutrient inputs.
4. We anticipate that a 20% or greater savings in feed costs, translating to ~10-15 % increase in on-farm profitability might occur with nutritional conditioning.
5. Key genes responsive to nutritional conditioning and associated with improved protein utilization will be identified for future optimization of fry feeding protocols or for selective breeding of tilapia.
6. Beneficial gut microflora associated with improved nutrient absorption may be identified that could be further developed as probiotics.
7. One graduate student and one postdoctoral fellow will receive training on sustainable farming practices and genomic technologies enabling aquaculture research.
8. Development of nutritional imprinting protocols that require lower protein diets for tilapia growout, will enhance tilapia production efficiency not only within the host country, but also may significantly benefit both the global and domestic (US) tilapia industry.

<u>Research Design and Activity Plan</u>

Location

Initial investigations will be performed the Grinnell's Fish Laboratory, North Carolina State University and pond investigations will be performed at the Fisheries Field Laboratory, Bangladesh Agricultural University providing the opportunity to extend advanced genomic capabilities to collaborating institutions within the host country (BAU).

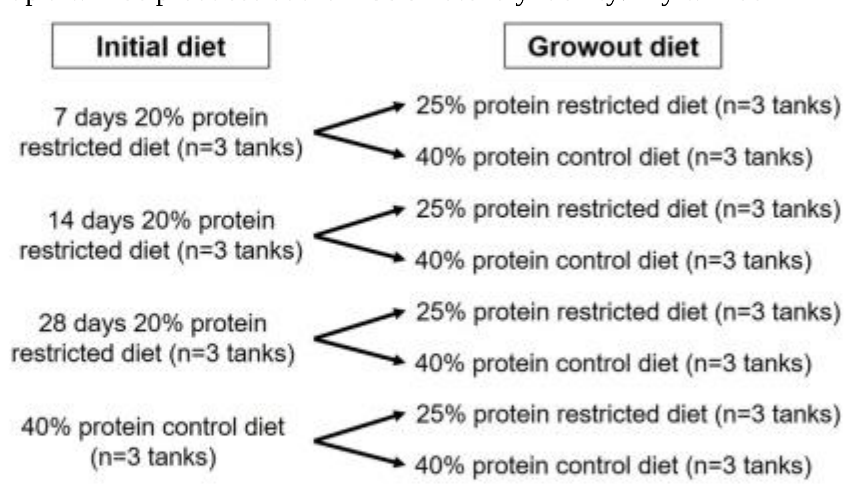
Methods

Experiment 1. Evaluate the effectiveness of nutritional conditioning on tilapia feed efficiency and production.

Null hypothesis 1: No significant differences in growth, feed efficiency or survival rates are observed by use of larval nutritional conditioning strategies for tilapia culture.

Null hypothesis 2: No significant differences in growth or economic parameters in pond-based culture are observed in nutritional conditioning of larvae.

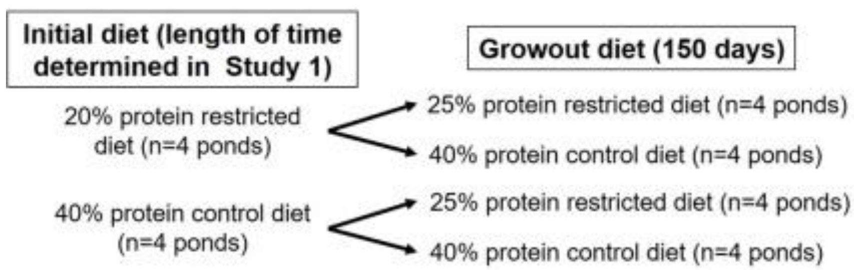
The aim of this investigation is to identify if nutritional conditioning of protein in post yolk-sac fry can lead to more efficient tilapia production. Restricted and normal protein diets will be produced at the NCSU feed mill with a nutritional profile as recommended by Mjoun et al., (2010). We will reduce the total dietary crude protein (while maintaining a constant amino acid profile, total energy) by 50% of the requirement from 40% to 20% during the developmental period immediately following yolk-sac absorption when first feeding occurs. Post yolk-sac Nile tilapia will be produced at the NCSU hatchery facility. Fry will be randomly assigned to one of 4 treatment group and restricted of protein for either 0, 7, 14, or 28 days and then subsequently fed a normal (40% crude protein; Mjoun et al., 2010; El-Sayed, 2006) or submaximal protein diet (25% crude protein) lower than the recommended level. This experiment will determine the longest restriction period for nutritional conditioning that will limit deleterious effect on fish survival and growth. Also by feeding fish a submaximal feed



of 25% crude protein it might be possible to achieve growth similar to those fish fed the recommended level (40%), but at substantially lower cost and with optimal utilization of the protein available (see Figure below). The control diet will have a nutritional profile as recommended by Mjoun et al., (2010). Each of the treatments will be grown in triplicate tanks (n=3; N=24).

The trial in tanks will be conducted for 120 days. Growth parameters (length and weight) will be monitored throughout the production period and for analyses of growth and adjustment of feeding rates. Feed rates will be fixed for all groups and will follow that used for tilapia production (15% bw/day for 0-15 g fish, 8% bw/day for 15-60 g fish, and 5% thereafter). Samples of tilapia anterior intestine tissue, fecal material from the intestine, and muscle tissue will be collected at 28 and 120 days from the time of first feeding for further analysis at NCSU (see Studies 2 and 3). Water quality parameters (*e.g.* ammonia, nitrites, nitrates, dissolved oxygen, and salinity) will be measured weekly. Temperature will be recorded daily. Specific growth rates (SGR) and feed conversion ratios (FCR) will be collected as production parameters. Growth and production (SGR, FCR) parameters for all treatments will be tested for significant differences using Analysis of Variance (JMP, SAS industries). The economic benefit of nutritional conditioning to production of tilapia will be determined from the amount and cost of feed needed to produce a kg of fish.

Should the nutritional conditioning tank trials in Experiment 1 work, we will conduct an additional experiment to evaluate the effectiveness of this technology in pond-based culture in Bangladesh for use by hatcheries and farmers for improving production efficiency of tilapia culture. Protein-restricted fry and growout feed will be produced by at a local feed manufacturer (CP or alternate) using the nutritional recommendations similar to Mjoun et al., (2010) and formulated by Dr. Ferket. Nile tilapia post yolk-sac fry will be obtained from a local hatchery and placed in hapas within a pond at BAU and fed a normal or protein restricted diet for a period determined from Experiment 1.



After this, control and nutritionally conditioned fry fish will be raised in hapas up to 2-3 g on a 25% or 40% crude protein diet. Fish will then be distributed to ponds and continued on the 25% or 40% crude protein diet for 150 days (N=16 ponds, 4 replicates/group; 100 m^2, 1.5 m depth ponds; 4 fish/m^2). All fish will be fed the respective diets on alternate days at a rate of 10 – 3% body weight/day. Our previous work shows that alternate day feeding produces of similar growth and yield as daily feeding (unpublished, current AquaFish report). All ponds will be fertilized weekly at 28 kg N and 5.6 kg P/ha. Differences in growth, yield, FCR among groups will be determined. A marginal benefit-cost analyses will also evaluate if there is a significant cost savings in rearing protocols that incorporate nutritional conditioning in tilapia.

Experiment 2. Identify key factors (gene networks) associated with enhanced nutrient utilization in response to larval nutritional conditioning in tilapia.
Null hypothesis 3: No significant differences in nutrient transporter mRNA abundance are observed in response to larval nutritional conditioning.

This investigation will identify those gene or gene networks that may be associated with elevated nutrient absorption in the Nile tilapia induced by nutritional conditioning (e.g. solute transporters, enzymes, immune factors, etc.) (Bröer, 2008). Standard RNA-Seq methods (Mortazavi et al., 2008) will be followed employing the Illumina HiSeq platform at the NCSU Genomic Sciences Laboratory (GSL). Samples (n = 8; N = 64) of tilapia anterior intestine (anterior). Expressed cDNA will be obtained from these tissues using established procedures (see Picha et al., 2006) and mRNA expression will be determined by RNA Sequencing (RNAseq). For each treatment, a bar-coded amplicon library (n=8) will be constructed, 2 pools will be made, and run on 2 Illumina lanes for each pool (125 bp, single end reads). This design will provide a depth of ~ 75 million reads for the total library. The NCSU Bioinformatics Consulting and Service Core (BCSC) will be utilized for the High Performance Computing (HPC) environment with the CLC Workbench license. Gene expression using Real-Time quantitative PCR (qPCR) will be performed on the most highly predictive groups of differentially expressed genes (DEG) identified in the RNAseq experiment to validate the effects discovered in the transcriptome analysis. Artificial neural networks will be generated from the DEG between treatments using WEKA software (Hall et al., 2009). The residuals of each highly significant DEG will be input into a Modulated Modularity Clustering program (Stone and Ayroles, 2009) to group the highly significant differentially expressed genes and Gene Ontology (GO) term enrichment of each module will determine cluster function using DAVID Bioinformatics suite (Huang et al., 2009).

Experiment 3. Identification of key microbial factors promoting increased nutrient absorption by enterocytes in the tilapia gastrointestinal tract.
Null hypothesis 4: No significant differences in microbial abundance or diversity occur with nutritional conditioning in tilapia.

This investigation will assess how gut microbial flora is altered by larval nutritional conditioning strategies, and will build on previous studies. Samples of tilapia fecal material will be collected from the tank experiment (see Experiment 1) and analyzed at NCSU. Samples collected from fish will be pooled together according to treatment group (n=6). We propose the use of a pooled sample design 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. Environmental (genomic) DNA will be isolated from fecal samples using procedures outlined in the 16S Metagenomic Sequencing Library Preparation protocol for the Illumina MiSeq system. Using universal bacteria/archeal primers (Fadrosh et al., 2014), the V3 and V4 regions of 16S ribosomal RNA sequence will be amplified for all microbial constituents present within the pooled samples. For each treatment combination, a bar-coded amplicon library (n=48) will be constructed and run on 2 Illumina lanes (300 bp, paired end reads). The sequencing on an Illumina MiSeq platform will be performed at the NCSU Genome Sciences Laboratory (GSL). The QIIME metagenomic toolkit (Caporaso et al., 2010) will be used for operational taxonomic unit (OTU) picking and core diversity analysis. The bioinformatics package PICRUSt (Langille et al., 2013) will be employed to predict microbial functionality of the identified bacterial communities. These predictions will be used to determine if certain bacterial community profiles are associated with increased feed conversion and utilization by nutritionally conditioned tilapia.

Trainings and Deliverables

1. The findings from Studies 1-3 will be reported through the Technical Reports of the AquaFish Innovation Lab (FIR).
2. The results will be presented in the scientific proceedings of the World Aquaculture Society Annual meeting or a regional Aquaculture conference.
3. We anticipate that novel findings arising from nutritional conditioning, intestinal transcriptome and metagenomic studies (Experiments 2-3) will likely lead to high-impact publications within the peer-reviewed literature and will be useful in the development of probiotic supplements. These papers will be produced following completion of the project.
4. One graduate student and one postdoctoral fellow will receive training on sustainable aquaculture farming practices, molecular mechanisms of growth in fishes, and/or genomic technologies enabling aquaculture research.

Schedule

August 2016 to February 2017: Experiment 1 Larval Conditioning Trial at NCSU.
November 2016 to December 2017: Quantitative analysis of the gut transcriptome (Experiment 2), identification of microbial gut populations by metagenomic analysis (Experiment 3).
April 2017 to December 2017: Pond-based Experiment 1.
January to February 2018: Final Technical Report.

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

AFRICA PROJECT: GHANA & TANZANIA
US Project PI: Kwamena Quagrainie, Purdue University
HC Project PI: Steve Amisah, Kwame Nkrumah University of Science & Technology

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

Collaborating Institutions and Lead Investigators

Sokoine University of Agriculture (Tanzania)	Sebastian W. Chenyambuga Nazael Madalla
Kwame Nkrumah University of Science & Technology (Ghana)	Regina Edziyie Nelson W. Agbo
Purdue University (USA)	Kwamena Quagrainie

Objectives

1. To develop cost effective diets promoting fast growth based on Moringa leaf, earth worm, and maggot meals as sources of protein for Nile tilapia diets.
2. To evaluate different cost minimizing feeding strategies.

Hypothesis

1. Growth performances of Nile tilapia fed diets containing Moringa leaf meal, earth worm meal, and maggot meal as sources of protein diets do no differ significantly
2. The effects of different feeding strategies on growth performance and survival of Nile tilapia are not significantly different.

Significance

Aquaculture is the fastest growing animal food-producing sector, with fish production increasing from less than 1 million tons per year in early 1950s to 66.6 million tons by 2012 with a farm gate value of US$ 137.7 billion and accounting for almost half of the world's fish food supply (FAO, 2014). Therefore, aquaculture plays a significant role in augmenting dwindling fish supply from capture fisheries due to over-exploitation and climate change impacts. It also offers great potential for food supply, poverty alleviation and enhanced trade and economic benefits (ADB, 2005). Smallholder aquaculture with commercial orientation can potentially be very profitable and the wealth created may be a powerful tool for poverty reduction (Wijkstrom and MacPherson, 1990). Aquaculture expansion in Asian countries like 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 the human diet. These nutrients include animal protein, essential fats, minerals and vitamins. Fish is a good source of essential long-chain omega-3 fatty acid docosahexaenoic acid (DHA) that is important for optimal brain and neurodevelopment in children and eicosapantaenoic acid (EPA) that improves cardio-vascular health. Thus, improving fish production from aquaculture will increase the availability of both macro- and micronutrients required in a healthy diet.

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 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 brans, 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} (Yi and Lin, 2001). For many decades, fishmeal and soybean have been used as the main sources of protein in fish feeds (El-Sayed, 1999; El-Saidy and Gaber, 2002). However, fish farmers in Tanzania are unable to afford good quality protein sources such as fishmeal, soybean meal, and other oil cakes that can meet protein requirement required for fast growth and development of fish. Thus, there is a need to identify cheaper alternatives 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 promotes higher growth rate of Nile tilapia, even better than soybean meal (Shigulu, 2012; Kitojo, 2013). Insects and other invertebrates have been shown to be cheaper sources of animal protein in tilapia diets (Omoyinmi and Olaoye, 2012). These invertebrates are abundant because of their short life cycle and ability to produce large numbers and high biomass within a short time. Our previous study showed that diets containing earthworm and maggot meals as sources of protein promote higher growth rate of Nile tilapia than the cotton seed cake based diets (Ally, 2015). Thus, the diets based on Moringa leaf meal, earthworm, and maggot meals seems to be appropriate alternative to conventional supplements as sources of protein as they have high crude protein content, abundantly available and affordable to small-scale farmers. However, these diets have not been tested under farmers' management conditions and the feed formulations have not been transferred to farmers. This study intends to transfer to small-scale farmers the technology of using Moringa, earthworm, and maggot meals as protein supplements to replace conventional fish meal and soybean meal. The aim is to enable the small-scale farmers to formulate better quality rations for Nile tilapia at an affordable cost from locally available feed ingredients.

Quantified Anticipated Benefits

The beneficiaries of this study will be various stakeholders involved in aquaculture, especially fish farmers and feed manufacturers. The expected outputs are as follows:

1. Two practical cost effective diets which promotes fast growth of tilapia developed, promoted, and adopted by fish farmers and feed manufacturers.
2. One best cost minimizing feeding strategy developed and adopted by fish farmers.
3. At least 40 fish farmers trained through workshops on feeding formulations and feeding strategies and adopt the use of the best diets and feeding strategy.
4. Two feed manufactures produce and sell the best diets developed by the project.
5. Five MSc students trained.

Research Design and Activity Plan

The study will be conducted on-farm and on-station using completely randomized design (CRD) to assign dietary and feeding strategy treatments to the experimental units.

Location

On-station studies will be conducted at the Aquaculture Research Facility, Department of Animal Science and Production, Sokoine University of Agriculture. On-farm experiments will be done in Morogoro, Dar es Salaam, Coastal, Mbeya and Iringa regions.

Methods

Experiment 1: Development of cost effective diets based on Moringa leaf, earth worm, and maggot meals as sources which promotes faster growth of Nile tilapia.

Practical and cost effective diets based on Moringa leaf, earth worm, and maggot meals as sources of protein in Nile tilapia diets will be developed based on previous studies by Shigulu (2012), Bazil (2014) and Ally (2015). These studies evaluated suitability of Moringa leaf meal, earthworm meal and maggot meal as sources of protein in Nile tilapia diets. Five diets will be formulated based on Moringa leaf meal, earthworm meal, and maggot meal and evaluated through growth trial in comparison with existing commercial fish meal based diet. Diets 1, 2 and 3 will contain Moringa leaf meal, earthworm meal and maggot meal as sole sources of protein in the diets, respectively. Diets 4 and 5 will contain a mixture of Moringa leaf meal and earthworm meal and Moringa leaf meal and maggot meal, respectively, in equal proportions. Hence, the total number of treatments will be six. The sixth treatment will be the control and the diet for this treatment will contain fish meal as the source of protein. All diets will have hominy meal, sunflower oil, wheat flour and mineral mix. All diets will be formulated to contain 30% protein and 10% lipid. Nile tilapia fingerlings will be stocked at a density of $3/m^2$ in outdoor concrete tanks of 4.5 m^2. The treatments (i.e., diets) will be allocated randomly to the tanks and each treatment will be replicated twice (PD/A CRSP, 1987, 2000). The fish will be fed at 5% of their body weight for a period of 120 days. Water quality parameters such as water temperature, dissolved oxygen, pH, total alkalinity, nitrate nitrogen, nitrite nitrogen, ammonia nitrogen and turbidity will be measured weekly to ensure that they are within acceptable limits. Body weight and length of individual fish will be measured at the start of the experiment and then every two weeks up to the end of the experiment. Weight gain, growth rate, specific growth rate, feed utilization efficiency as well as cost effectiveness of the diets will be determined and the best diets will be identified.

Experiment 2: Development and evaluation of cost minimizing feeding strategies
Development of feeding strategies will be based on works by Kitojo (2013) and Hasan & New (2013). Effect of feeding levels (2.5% vs 5% of body weight) and restricted feeding (daily feeding vs alternate day feeding) will be tested using the best diet identified in experiment 1 above. Nile tilapia will be stocked at a density of $3/m^2$ in outdoor concrete tanks and the growth trial will last for a period of 120 days. Water quality parameters such as water temperature, dissolved oxygen, pH, total alkalinity, nitrate nitrogen, nitrite nitrogen, ammonia nitrogen, and turbidity will be measured weekly to ensure that they are within acceptable limits. Body weight and length of individual fish will be measured at the start of the experiment and then every two weeks up to the end of the experiment. Weight gain, growth rate, specific growth rate, feed utilization efficiency as well as cost effectiveness of the diets will be determined and the best diets will be identified.

Sensitization of fish farmers and feed manufacturers to adopt the best feed formulations and feeding strategies for Nile tilapia
The best diets and feeding strategies from experiment 2 will be evaluated in an on-farm trial in two villages in Morogoro region and two villages in Iringa region. Morogoro has warm climate while Iringa has a cold climate. In each village three farmers with at least two fish ponds will be purposely selected based on the willingness to participate in the trial. The farmers' ponds will be drained, dried, limed and filled with water to a depth of 1 m before the start of the experiment. All ponds will be stocked with Nile tilapia at a stocking density of $3/m^2$. For each farmer two ponds will be used, one pond will be under the best diet and feeding strategy identified in experiments 1 and 2 above and the second pond will be under normal farmer's feeding practice. The on-farm experiment will last for 180 days. Water quality parameters such as water temperature, dissolved oxygen, pH, total alkalinity, nitrate nitrogen, nitrite nitrogen, ammonia nitrogen and turbidity will be measured at monthly intervals. Body weight and length of individual fish will be measured at the start of the experiment and then every month up to the end of the experiment. Weight gain, growth rate, specific growth rate, feed utilization efficiency of fish under the two treatments will be compared.

Trainings and Deliverables

A workshop will be conducted to train farmers to formulate the best diets identified in experiments 1 and 2. During the workshop the farmers will be sensitized to use the diets to supplement fish grown in their ponds. The aim of this part will be to enable the farmers to formulate better quality supplementary diets for feeding their fish. In addition, selected feed manufacturers will be visited in Dar es Salaam, Morogoro, Iringa, and Mbeya regions and sensitized to use the best feed formulations developed in the experiments 1 and 2. The sensitization will involve sharing of the results of experiments 1and 2 with the feed manufacturers and promotion of the developed feed formulations and feeding strategies through distribution of brochures. In summary:

1. At least 40 fish farmers trained through a workshop on feeding formulations and feeding strategies and adoption of the best diets and feeding strategy.
2. Five MSc students trained.

Schedule

Activities	2016						2017												2018	
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Objective 1: Development diets based on Moringa leaf, earth worm and maggot meals as sources of protein																				
Activity 1.1: Collection of feed materials	■																			
Activity 1.2: Formulation of six diets based on Moringa, earthworm, maggot and fish meals as sources of protein in the diets	■																			
Activity 1.3: Conducting on-station growth trial to evaluate the six diets		■	■	■	■															
Activity 1.4: Data analysis and report writing						■														
Objective 2: Development and evaluation of cost minimizing feeding strategies																				
Activity 2.1: Collection of feed materials and formulation of the best diet identified in objective 1						■														
Activity 2.2: Conducting on-station growth trial to evaluate cost minimizing feeding strategies							■	■	■	■										
Activity 2.3: Data analysis and report writing											■									
Activity 2.4: Selection of farmers for testing the best diet and feeding strategy on farm											■									
Activity 2.4: Conducting on-farm growth trial to evaluate the best diet and feeding strategy												■	■	■	■	■	■			
Activity 2.5: Production of brochures																		■		
Activity 2.6: Conducting farmers' Training workshops on feed formulation and feeding strategies																		■		
Activity 2.7: Sharing of research results with feed manufacturers																			■	
Activity 2.5: Sensitization of fish farmers and feed manufacturers on the developed feed formulations and feeding strategies for tilapia																			■	
Activity 2.8: Report writing																			■	
FINAL REPORT WRITING																				■

TOPIC AREA

CLIMATE CHANGE ADAPTATION: INDIGENOUS SPECIES DEVELOPMENT

❖

SUSTAINABLE SNAKEHEAD AQUACULTURE IN CAMBODIA

ASIA PROJECT: CAMBODIA & VIETNAM
US Project PI: Robert Pomeroy, University of Connecticut – Avery Point
HC Project PI: So Nam, Inland Fisheries Research and Development Institute

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND01UC

Collaborating Institutions and Lead Investigators

University of Connecticut-Avery Point (USA)	Robert Pomeroy
Inland Fisheries Research and Development Institute (Cambodia)	So Nam Nen Phanna Chheng Phen
Can Tho University (Vietnam)	Tran Thi Thanh Hien

Objectives

1. To compare growth performance and survival rate of different snakehead strains regarding to weaning and grow-out experiments.
2. To compare economic efficiency of grow-out experiments of the different snakehead strains.

Significance

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 costs and the main type of feed for wild snakehead culture was small-sized fish (SSF). 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 Vietnam. Snakehead is found in most Cambodian and Vietnamese dishes at all wealth class levels (i.e., from poor, medium to rich people). During the first phase of AquaFish CRSP (2007-2009), the study revealed that 33 freshwater SSF in the Vietnam Mekong Delta (Hien et al., 2015) and nearly 200 SSF in the Cambodia Mekong basin (So Nam 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.

The government of Cambodia put a ban on snakehead farming in September 2004 by Announcement No. 4004. After the ban on snakehead culture in Cambodia, snakehead have illegally been imported from the neighboring countries, particularly from Vietnam, to supply high local market demand in Cambodia. Furthermore, freshwater SSF have illegally been exported to Vietnam for feeding the significant and commercial snakehead aquaculture industry (So Nam et al., 2009). 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. These snakehead fish farmers have lost their important livelihoods and household income. Moreover, the ban also did 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 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 guidance for farmers. This investigation will provide for this research based guidance on weaning and grow-out of snakehead.

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 hatching could gradually and successfully accept AquaFish CRSP Snakehead Formulated Feed developed by the AquaFish CRSP project as a replacement for SSF at the rate of 10% every three days for a period of 30 days of feeding. During the third phase of AquaFish Innovation Lab (2013-2015), the wild snakehead larvae aged 17 days old after hatching could be gradually weaned on formulated feed (using the protocol adopted from Hien and Bengtson 2009; 2015. These fish were grown from larvae to adult fish (as F_1 generation) at FARDeC by feeding them with pelleted feed developed by AquaFish CRSP project (Nen et al., 2015). F_1 mature adult snakehead will be developed into F_2, F_3 and F_4 generations to optimize the growth and survival rate while feeding on formulated or pelleted feed to ensure the efficient production and economic benefits 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 will conduct a breeding experiment at CTU between wild and domesticated snakehead collected in Vietnam. If the offspring 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.

With the above initial results, the Ministry of Agriculture, Forestry and Fisheries announced the release of the ban on snakehead aquaculture in Cambodia in April 2016. At the same time, the Fisheries Administration formed a Technical Working Group to discuss an Action Plan and Technical Guidelines for sustainable development of snakehead aquaculture in Cambodia.

This research will provide technical support to Cambodia's Fisheries Administration for the formulation of an appropriate action plan and technical guidelines to adequately develop a sustainable snakehead aquaculture industry in Cambodia without doing harm to the wild fisheries on which so many people depend. In addition, this research will also contribute to the goal of the overall project, which is to enhance trade and investment for global fishery markets and improved nutrition and food security through sustainable aquaculture development in Cambodia, especially in the context of the recently released ban on snakehead aquaculture. There is a great potential for enhanced trade in snakehead fresh and processed forms and investment for snakehead in Cambodia after the ban released. Moreover, if the wild snakehead fisheries are 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 Vietnam, 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 will focus on optimization of the domestication and development of F_1 into F_2 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 will also be conducted in a purpose to provide technical and policy recommendations for sustainable snakehead farming in Cambodia.

Quantified Anticipated Benefits

This research will provide information on domesticated breeding, weaning and grow-out of snakehead fish, especially development of Cambodia's snakehead aquaculture technologies, in order to support the lifting of the ban on snakehead culture in Cambodia. The following are quantifiable anticipated benefits:

- At least 20,000 farmers in Cambodia will benefit from this Investigation by restarting their snakehead culture leading to increased household income and improved snakehead fish market and trade.
- 250 scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staff, and private sector working on the issues of snakehead aquaculture in Cambodia as well as in other Mekong riparian countries will be better informed about research methods and findings, and have better recommended policies and strategies for sustainable snakehead aquaculture.
- Two (under) graduate students will be supported and trained by this investigation through their B.Sc./M.Sc. thesis research.
- At least 1,000,000 people who consume snakehead fish in Cambodia and other Mekong riparian countries will receive indirect benefits from this research.
- Benefits to the US include improved knowledge and technologies on domestication breeding, weaning and growth out of snakehead and this aquaculture is considered as a climate change adaptation measure. It will also allow Dr. Pomeroy to continue and expand his research program on economics and policy of fisheries and aquaculture in Southeast Asia.

Research Design and Activity Plan

Location

All weaning and grow-out trials will be 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), which has many broodstocks, breeding and weaning earthen ponds, hapa-nets and cement tanks, a small fish feed mill for fish pellet production and laboratories.

Methods

Null hypothesis: No differences among larvae are produced by the following snakehead broodstocks: current F1strain, Tonle Sap wild strain, Mekong River wild strain and Vietnamese domesticated striped snakehead.

(a) **Collection of brood snakehead fish:** In addition to available breeders developed at FARDeC, adult/mature wild snakehead (*C. striata*) will be collected from six different natural water bodies in six provinces of Cambodia (i.e. Kampong Chnang, Pursat, Siem Reap, Kampong Cham, Kandal and Prey Veng provinces) and conditioned at FARDeC hatchery, Cambodia in order to find the best strain for the highest growth rate or disease resistance. This program will contribute to the broodstock development in the near future and also preserve a genetic pool of hatchery broodstock native to Cambodia. It is doable and there is enough time to achieve this task because the first maturation age of the striped snakehead *Channa striata* is about one year. Frequent use of imported domesticated stocks of snakehead from Vietnam carry pathogens that could spread to the Cambodian wild snakehead and other fish stocks according to the study on snakehead disease and water quality conducted in the second phase of AquaFish project (2009-2011) (Pham Minh Duc et al., 2011). Domesticated snakehead (having already adapted to pellet feed) will be purchased from three different hatcheries in three provinces of the Mekong Delta in Viet Nam: Dong Thap, An Giang and Can Tho/Vinh Long provinces, quarantined to assure that pathogens are not being introduced, and also conditioned at FARDeC for induced spawning to produce larvae to serve as the control for the experiment.

(b) **Induced spawning using HCG hormone:** All snakehead broodfish will be checked monthly for egg maturation based on the method of Nikolsky (1963). Broodfish with egg maturation at the same stage will be selected for induced spawning in order to simultaneously produce batches of larvae for the weaning experiment. HCG hormone will be used for induced spawning using the best dose obtained from So et al., (2011) in the final technical report during AquaFish CRSP phase 2.

(c) **Weaning experiment:** Comparison of survival and growth rate of larvae/fingerling from several broodstocks: current F1strain, Tonle Sap wild strain, Mekong River wild strain and Vietnamese domesticated striped snakehead. The larvae of all above brooders will be weaned at the same time using the same weaning protocol for *C. striata* adapted from Nen Phanna et al., (2015), So Nam et al., (2011) and Hien and Bengtson (2009; 2011) from live *Moinasp.* to formulated feed.

c.1) The protocol: After yolk absorption at 3 days after hatching (dah), larvae will be fed with live *Moina* for 7 consecutive days till 10 day-old larvae (dah), and then larvae will be 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 day-old larvae (dah). We will start weaning *Channa striata* larvae on formulated feed at 17day with replacement of trash fish by 10% per day until freshwater small-sized fish will be completely replaced by formulated feed. Formulated feed contains 40-50% crude protein.

c.2) Experimental set-up for weaning: Each of the four treatments will have five replicates. Larvae will be stocked in 50-L tanks with stocking density of 5 fish/L in recirculating water system. The fish will be fed to satiation by hand twice daily. Any remaining feed and feces will be siphoned out before feeding. Fish mortality, food consumption, and water quality (temperature, pH, and dissolved oxygen) will be recorded daily. Larvae will be sampled, weighed and measured at biweekly intervals. At the end of the experiment, final body weight (FBW, mg) and wet weight gain (WWG, mg) including survival rate will be determined. The experiment will last for 30 days.

c.3) Statistical analysis: Difference among the treatments on growth and survival rate and feed intake of snakehead larvae will be determined by one- way ANOVA. Tukey's HSD test is used to determine specific differences among means where appropriate.

(d) **Grow-out diet experiments:** Comparison of grow-out performance for the same strains used in the weaning experiments. Successfully weaned-larvae/fingerling in each treatment above will be moved to continue grow-out in hapas allocated in four earthen ponds ($300m^2$) to continue compare their growth performance and survival rate and to see their feeding adaptation on pelleted feed.

d.1) Experimental set-up for grow-out on pelleted feed: There are four treatments (F1 strain, wild Tonle Sap strain, wild Mekong River strain, and Vietnamese domesticated strains). Each treatment will have five replicated hapas (5x3x1.5m), and allocated separately in one individual earthen pond. A stocking density will correspond to the fish remaining in each treatment from the weaning experiment. The fish will be fed with commercial pelleted feed (45% CP) to satiation by hand twice daily at 09:00h and 16:00h. The amount of feed used and fish mortality will be recorded daily. Water quality parameters (Secchi disk transparency, temperature, pH, dissolved oxygen, NH_3, and NO^-_2) will be monitored weekly. Growth will be measured monthly by weighing 30 fish/hapa. The survival rate will be determined at the end of experiment. The experiment will last for 6 months.

d.2) Cost and benefit analysis will also be conducted to determine economic returns and efficiencies of *C. striata* cultured on pelleted feed by the following formulas:

-Total Revenue = Quantity * Price/unit
-Total Cost =Variable Cost + Fixed Cost
-Net return = Total Revenue – Total Cost
-Economic Efficiency = Total Revenue/Total Cost

The analysis will be based on farm-gate prices in Cambodia for harvested *C. striata* and current local market prices for all other items (e.g. cost of the pelleted feed; hapa net) and will be expressed in US dollars (US$).

d.3) Statistical analysis Difference among the treatments on growth and survival rate and feed intake of snakehead larvae including economic return will be determined by one-way ANOVA. Turkey's

HSD test is used to determine specific differences among means once the ANOVA indicates that significant differences are present at p= 0.05 using SPSS 16.0.

(e) **Back-crossing experiment in Vietnam:** Wild broodtock collected from Camau conservation park and domesticated broodstock bought from a hatchery in Dong Thap or An Giang province, Vietnam will be held in the same hatchery conditions at CTU for at least one month before we induce reproduction through hormonal injections. Four crosses will then be carried out: (i) wild snakehead male and female (WxW); (ii) domesticated snakehead male and female (DxD); (iii) wild male and domesticated female (WxD); and (iv) domesticated male and wild female (DxW). Fry from these crosses will be separately reared in tanks (for 1-2 month) through weaning under the same conditions. Each cross will have three replicate tanks Fish will be reared using the standard CTU procedures, i.e., live feed until weaning to formulated feed in the hatchery water quality will be monitored for temperature, dissolved oxygen, ammonia and nitrite. Fish performance evaluation will include survival and growth rates in the hatchery Data will be analyzed using one-way analysis of variance followed by Tukey's HSD test where appropriate. The null hypothesis is that no differences exist in fish performance among offspring from the four crosses.

Trainings and Deliverables

Short-term training

On-station training on domestication breeding, weaning, grow-out and feed formulation technologies of the striped snakehead *Channa striata* obtained from first and second phases of AquaFish CRSP and third phase of AquaFish Innovation Lab will be conducted in the fourth quarter of 2017 at FARDeC for Cambodian fish farmers and government fisheries officers who are working at seven provinces where wild snakehead breeders and fin clips are being collected for the domestication and genetic diversity assessment by IFReDI.

Long-term training

One undergraduate (BSc) and one graduate (MSc) students will be partially supported for their thesis work under this investigation.

The deliverables of this investigation will include (1) a final technical report, including policy recommendations, and (2) a factsheet.

Schedule

The duration of implementation of this proposed investigation will be 24 months, starting from 1 March 2016 and ending 28 February 2018.

This investigation is planned to be implemented as below:

Activity	Beginning	Ending
Collection of wild or non-domesticated broodfish	March 2016	April 2016
Collection of domesticated broodfish	March 2016	April 2016
Experimental designs	April 2016	May 2016
Implementation of the experiments	June 2016	July 2017
Analysis of results and report preparation	August 2017	December 2017
Conduct of on-station training on domestication breeding, weaning, grow-out and feed formulation technologies of the striped snakehead *Channa striata*	October 2017	October 2017
Consultation and dissemination workshop on research findings	January 2018	February 2018
Finalization of final technical report	January 2018	February 2018

TILAPIA AND KOI (CLIMBING PERCH) POLYCULTURE WITH *PANGASIUS* CATFISH IN BRACKISH (HYPOSALINE) WATERS OF SOUTHERN BANGLADESH

ASIA PROJECT: BANGLADESH
US Project PI: Russell Borski, North Carolina State University
HC Project PI: Shahroz Mahean Haque, Bangladesh Agricultural University

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND02NC

Collaborating Institutions and Lead Investigators

North Carolina State University (USA)	Russell Borski
Patuakhali Science and Technology University (Bangladesh)	Zahid Parvez Sukhan
	Md. Lokman Ali
	Shahroz Mahean Haque

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.

Significance

The focus of this investigation is 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 are rising 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; 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 to 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 in 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 (*Oreochromis 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). Its production is primarily limited to freshwater environments. Tilapias 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). The 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 Bangladeshi's (Apu, 2014). It is a hardy, air-breathing fish capable of living in low oxygen environments (Hasan et al., 2007). Recent evidence suggests Koi larvae can be raised in low saline 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 will assess the first time the potential of culturing Koi to market size in brackish water ponds.

Pangasius catfish was introduced to Bangladesh in 1990's, and since then it has become a thriving aquaculture industry with over 300,000 tons 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 tons / 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 aquaculture to these coastal communities, whose water resources are largely underutilized, could enhance the dietary consumption of protein for low-income families, as well as provide new sources of income and employment in an 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 with freshwater pond culture (Ali et al., 2015). 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.

In the proposed studies we wish to expand 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, 2006). There 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 will first assess the growth and production of tilapia and Koi alone and together in polyculture with *Pangasius* in hyposaline brackish water ponds and will then assess if increasing their density might further improve fish production. It is anticipated if *Pangasius*-tilapia-Koi polyculture can be achieved in the Southern coastal region of Bangladesh, the production levels of these fishes could effectively double, which would significantly impact the diet and economic viability of coastal communities.

Quantified Anticipated Benefits

1. We anticipate that Koi can be cultured to market size in hyposaline, brackish water ponds.
2. Polyculture of tilapia and Koi with *Pangasius* catfish will improve economic returns over *Pangasius* monoculture alone.
3. Increasing the stocking densities of Koi and/or tilapia relative to *Pangasius* will enhance efficiency and return on investments in polyculture production of the three species.
4. Polyculture of tilapia, Koi and *Pangasius* catfish in brackish waters of Southern Bangladesh has the potential to double annual aquaculture production and will increase the diversity of aquaculture species produced and the dietary nutrition available to farming households.
5. We anticipate significant improvements in environmental water quality in the tilapia and koi polycultured with *Pangasius*.
6. We anticipate that successful development of this project will increase livelihood options and better food-security for low-income families impacted by overfishing and rises in sea level (global climate change).

Research Design and Activity Plan

Location

These studies will be performed in ponds of participating farmers in the Pauakhali district of the greater Barishal region of Southern Bangladesh.

Methods

Experiment 1. Evaluate if freshwater Koi (climbing perch) can be successfully cultured in seawater-encroached hyposaline waters of coastal Southern region of Bangladesh. Assess production performance and economic impacts of tilapia and Koi polycultured with Pangasius in brackish waters.

Null Hypothesis 1: No differences in growth efficiency, water quality, yields or economic returns are observed with Pangasius cultured together with koi or tilapia or both.
Null Hypothesis 2: No differences in growth efficiency, water quality, yields or economic returns are observed with Pangasius cultured alone versus Pangasius polycultured together with either koi, tilapia or both.

Based on our previous work we showed that *Pangasius* can be cultured in salinities as high as 10-12 ppt and that growth and production yield is similar to that of fish cultured in 5-8 ppt or in 0-0.5 ppt (freshwater). We also found that increasing the density of *Pangasius* to 3 fish/m^2 increased overall yield of fish, but caused only a mild change in return on investment. Here we will utilize 4 fish/m^2 in monoculture and test if addition of Nile tilapia or Koi alone or a combination of the two with *Pangasius* might enhance the efficiency and economic return of fishes in polyculture (fish densities will be similar for all groups). This experiment will also establish if Koi can be grown to market size in hyposaline waters. All fish will be cultured at a range of 5-8 ppt, endemic to the coastal region in Barishal and that reflects extrinsic fluctuations in salinity across the growing season.

The experimental design is as follows:

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Pangasius (fish/decimal)	160 (4.0/m^2)	80 (2.0/m^2)	80 (2.0/m^2)	80 (2.0/m^2)
Tilapia (fish/decimal)	-	80 (2.0/m^2)	-	40 (1.0/m^2)
Koi (fish/decimal)	-	-	80 (2.0/m^2)	40 (1.0/m^2)
Salinity range (ppt)	5-8	5-8	5-8	5-8
Feeding	Std. regime	Std. regime	Std. regime	Std. regime
Replication	4	4	4	4

Ponds will be selected on the basis of the surface water salinities from participating farmers in Patuakhali district. WorldFish has aided us in selection of farmers and will continue to do so. We will target women in our studies and anticipate that >50% of the pond operators will be women based on our previous studies. Ponds of ~400 m^2 will be dried, eliminated of unwanted species and then limed at 2.5 g/m^2 with CaCO3 according to standard practices. They will be filled with adjacent surface water and fertilized one week prior to stocking (28 kg N; 7 kg P/ ha).

Pangasius, Koi, and sex-reversed Nile tilapia fingerlings (~10 g) will be stocked in all ponds for a 160-day growout period. Fish will be fed a Mega floating feed (28% CP, 7% Fat; Spectra Hexa Feeds, Ltd.) at the rate of 10% of bw/day down to 3% bw/day. Feed will be applied twice daily at 9.00 and 16.00.

Fortnightly subsampling of the experimental fish will be done using a cast net to measure growth performance of experimental fish and to adjust feeding rate. After completion of the growth trial, a subsample of fish weights and lengths and the total number of fish harvested for each pond will be determined for assessing survival rates, total yield, and final weights and lengths.

Water quality parameters including water temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrite, and salinity will be measured fortnightly.

Production parameters including % weight gain, specific growth rate, and feed conversion will be calculated. Production yields (market weight, kg), estimated market returns, feed input costs (feed, fertilizers, labor, fingerlings), and labor costs will be gathered for all treatment groups at the end of experiment for marginal cost-benefit analysis.

All treatments will be tested for significant differences in growth (mean length, weight X time), growth efficiency (specific growth rate, feed conversion ratio), yield and water quality using Analysis of Variance ($p < 0.05$; preplanned contrasts).

Experiment 2. Assess effect of increased stocking density of tilapia and Koi in brackish water Pangasius-Koi-tilapia polyculture.
Null Hypothesis: No improvement in growth, water quality, yield or economic returns is observed by increasing the proportion of tilapia, Koi or both in polyculture with Pangasius.

We expect that that polyculture of either tilapia (Treatment 2, T2), or Koi (Treatment 3, T3) or both (Treatment 4, T4) with *Pangasius* from Experiment 1 will enhance economic returns over *Pangasius* monoculture, primarily because of the higher value of tilapia and Koi. It is also likely that tilapia will utilize the natural productivity in ponds, which could increase overall yields or food conversion efficiency while improving water quality. We will take the best result from Treatments 2-4 of the Experiment 1, and conduct a second experiment to determine if increasing the proportion of either tilapia, koi, or both relative to *Pangasius* might further improve production efficiency and economic returns. Ponds (N = 15, 5 replicates per treatment) from operators used in Experiment 1 will be used. Total stocking densities of fishes will remain the same among groups, but the proportion of tilapia or Koi or their combination will be increased. The experiment design is as follows:

Parameter	Treatment 1	Treatment 2	Treatment 3
Pangasius (fish/decimal)	100 ($2.0/m^2$)	80 ($1.5/m^2$)	60 ($1.0/m^2$)
Best of T2-T4 in Experiment 1 (fish/decimal)	80 ($2.0/m^2$)	100 ($2.5/m^2$)	120 ($3.0/m^2$)
Salinity range (ppt)	5-8	5-8	5-8
Feeding	Std. regime	Std. regime	Std. regime
Replication	5	5	5

The preparation and fertilization of ponds and feeding regimen for fishes will be performed as described in Experiment 1.

Growth and water quality measurements will be collected as described previously, with food amounts recorded daily for economic analysis. All treatment groups will be tested for significant differences in growth (mean length and weight), growth efficiency (specific growth rates, feed conversion ratio), and water quality using ANOVA. A marginal cost-benefit analysis will be determined incorporating total production yields (kg), expected market returns, feed and labor costs for these treatments.

Trainings and Deliverables

1. Two students will receive training on tilapia and koi polyculture with *Pangasius* in hyposaline waters and its economic impacts as part of their MSc thesis work.
2. The findings from these studies will be documented through the Technical Reports of the AquaFish Innovation Lab (FIR).
3. The work will be presented at Patuakhali Science & Technology University workshop (in Investigation 5), and may also be presented at a regional Aquaculture Conference.
4. If the technologies for tilapia-koi polyculture with *Pangasius* in hyposaline prove effective, then results will also be disseminated through production of an extension factsheet in the local language for wider outreach to farmers, extension agencies of the government, and NGOs (in Investigation 5). A paper reflecting the results should they be promising will also be prepared for publication in a peer-reviewed journal.

Schedule

June 2016 to February 2017: Experiment 1; Assess production performance and economic impacts of tilapia and Koi polycultured with *Pangasius* in brackish waters. Complete analyses and preparation of report.

April 17 to December 2017: Experiment 2; Effects of stocking density on growth performance of *Pangasius* and Tilapia/ Climbing perch in hyposaline water polyculture pond.

January 2018 to February 2018: Complete Final Technical Report and begin preparing manuscript.

DEVELOPMENT OF CAPTIVE BREEDING, LARVAL REARING TECHNOLOGIES AND MANAGEMENT PRACTICES FOR AFRICAN LUNGFISH (*PROTOPTERUS AETHIPICUS*)

AFRICA PROJECT: KENYA & UGANDA
US Project PI: Joseph J. Molnar, Auburn University
HC Project PI: John Walakira, National Fisheries Resources Research Institute

Climate Change Adaptation: Indigenous Species Development/Experiment/16IND03AU

Collaborating Institutions and Lead Investigators

Auburn University (USA)	Joseph J. Molnar
	Jeffrey Terhune
	Claude Boyd
North Carolina State University (USA)	Benjamin Reading
	Russel Borski
National Fisheries Resources Research Institute (Uganda)	John Walakira

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.

Significance

The general objective of this study is to generate information on aspects of reproductive biology, larval rearing and growth of *Protopterus aethiopicus* for sustainable production in captivity while improving nutrition and household incomes for local populations in Uganda and the neighboring regions. The studies and experiment continue the program of captive reproductive process determination and genetic description accomplished in the previous cycle of research.

The African lungfish (*P. aethiopicus)* is one high value species in great demand within the lakes region. 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, to best 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.

Quantified Anticipated Benefits

- Basic guidance on production and management of lungfish expressed in a farmer-oriented leaflet.
- Basic nutrition profile of lungfish grow out expressed in a technical report for extension.
- Larval weaning protocol for the fish established and expressed in a manual.
- Basic fingerling supply and grow out information published in a journal article.
- Inform the merit of continuing research into developing low-cost, artificial breeding technologies for these species.

Research Design and Activity Plan

Location

Kajjansi, Uganda.

Methods

Experiment 1: Assess the seasonal reproductive cycle of the African lungfish 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. Apparently, 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.

*1.1 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 will be collected, frozen at -20^{o} C, and shipped to North Carolina State University for steroid assays. The steroid levels will be 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) will be 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 (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* will be 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) will be employed to test differences in various reproductive parameters over time. The study will be conducted for 12 months.

1.2 Gonad characteristics. Somatic index (GSI), condition factor, length at maturity, fecundity and sex ratios will be measured. Samples from Lakes Wamala and Bisina will be collected monthly for a year, weighed and dissected to expose gonads for staging and gender identification. Standard histological methods (Bancroft and Gamble 2002) will be conducted to aid confirmation of sex of gonads (especially tiny gonads) and stages. Digital photographs of the gonad histology will be taken.
Gonads will be staged following Brown-Peterson et al., (2011) and Wallace & Selman (1981) classification and the mean diameter of the top clutch of oocytes will be 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 will be determined by analysis of variance (ANOVA). Fecundity will be determined by gravimetric method (Kipling and Frost, 1969). Significant deviation from the hypothetical 1:1 ratio of monthly and class size sex ratios will be determined using a chi-square test and binomial probability.
Sexual maturity will be 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.

1.3 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.

1.4 Sample collection, morphometric, and gender identification. African Lungfish all size classes will be collected from Lakes Wamala and Bisina system and measured for total length and weight. Approximately 40-60 fish will be collected using locally available harvesting gears every month. Fish will be anesthetized with tricaine methane sulfonate (MS-222) buffered with 0.2 ml $NaHCO_3$, pH = 7. Morphometric parameters will be 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) will be characterized using digital images to determine the differences among wild populations of the two lakes. Data will be subjected to statistical analysis as described by Mir et al., (2013) to evaluate morphological differences. Fish samples will be dissected to identify individual sex, which will be correlated to phenotypic observations based on existing scientific knowledge.

1.5 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 will be used to aspirate gonadal tissue. Fish will be anesthetized and oocyte staging of biopsied females will be conducted as described above. Additionally, digital photographs of the oocyte stages will be compiled with images collected in **1.2 Gonad characteristics** (above) to form a guide for African lungfish ovarian development.

1.6 SNP panel for gender identification. Molecular genetic markers will be identified to ascertain and explain sex differentiation and determination of African lungfish since this information is apparently unknown. Validation will be 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 will be 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 will be extracted using a Trizol protocol and stored at -80 °C. Libraries for RNA-seq will be 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 will be identified using Trinity/v2.0.6 software. The total number of SNPs detected will be selected based on the SNPs expected heterozygosity and the Polymorphic information content (PIC) as a tool for genetic diversity.

DNA will be prepared using protocols described by Sambrook and Russell (2001). DNA will be extracted using proteinase K and phenol-chloroform and amplified using polymerase chain reaction (PCR) primers. A complete mtDNA sequence of P. *dolloi* will be used to develop primers as described by Zardoya and Meyer (1996).

Following the Peukert et al., 2013 approach, genomic polymerase chain reaction (PCR)-amplification will be 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 will be 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 will be re-sequenced. Positions of 3’- and 5’-UTR, introns and exons will be determined using GeneSeqer.

Using Ansmann et al., (2012) methods, 20 males and 20 females whose sex is phenotypically determined will be verified using the SRY/ DMRT1 genes. Fragments of SRY/ DMRT1 genes will be amplified using PCR with 20–25 ng DNA, 0.15 M of each primer. The PCR profile will be 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 will be separated on agarose gel to determine sex based on length differences

Experiment 2: Identify breeding technologies for producing African lungfish seed.
*2.1 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 will be developed. Simple and low-cost breeding technologies will be 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 will be subjected to simple artificial reproduction techniques to evaluate the working fecundity, egg survival and hatchability.

Larval rearing will be 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 will be 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).

*2.2 Identify efficient artificial breeding technologies for African lungfish***.** Modifying protocols used by Vijaykumar et al., (1998), mature broods will be conditioned for 3-4 weeks in concrete tanks at NaFIRRI facilities, and will be 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 include the administration of:

2.2.1 Gonadotropin releasing hormone analogue (GnRHa) implants. 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 will be 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).

2.2.2 Human chorionic gonadotropin (HCG) or Chorulon. At an application rate of 2.0 mL per 5 kg fish (330 IU/kg hCG to male and female broods, according to Hodson and Sullivan (1993).

2.2.3 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 will examine fecundity, hatchability and survival of post-hatchlings. Water quality parameters will be monitored weekly to understand environmental factors affecting captive breeding. Best approaches will be selected based on statistical analysis of factors that produce better quantity, viability, and quality of lungfish spawns.

2.3 Compare breeding methods of African lungfish in captivity:
2.3.1 Manipulated environment breeding. Selected mature broodfish (males and females) will be 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 will be manipulated to simulate a flood pulse to promote natural ovulation, spawning, and fertilization. Fecundity, hatchability and survival of post-hatchlings will be evaluated.
2.3.2 Evaluating hormone induced fish. Two approaches will be used. First, the fish will be 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 will be used to analyze the egg and larval mortalities and differences in means will be evaluated by analysis of variance (ANOVA) or non-parametric analog tests. Water quality parameters—pH, alkalinity, temperature—will be monitored, weekly, to understand environmental factors affecting artificial breeding. The two approaches will be 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 will be 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 will be evaluated for their performance on survival and growth of the larvae. Larvae from induced spawning experiments will be 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) will be given to the larvae in 4 replicates. The larvae after hatch will be raised until the juvenile stage. Sampling will be done at intervals to record the weights and lengths of the fish. The analysis of variance (ANOVA) will be 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 tests will be 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 will be randomly allocated into six tanks and a commercial feed of varying crude protein content (35% and 40%) will be evaluated to establish its performance on the growth of *P. aethiopicus*. The same experimental arrangement will be evaluated in ponds at the research station and with some farmers (on farm).
Fish will be 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 will be determined by analysis of variances (ANOVA). The experiment will be conducted for 120 days. All the fish will be harvested, counted and weighed individually. The increase in length and weight will be

calculated from: final - initial length or weight. Survival rate will be calculated on the basis of the number of fish harvested from the formula:

$$\text{Survival rate} = \frac{\text{No of fish harvested}}{\text{No of fish stocked}} \text{ X } 100\%$$

- Gross production will be determined from the expression;
- Gross production = (Average final weight of fish X Total no of harvested fish)
- Net production will be determined from the expression;
- Net production = (Average final weight increase of fish X Total no of harvested fish) kg

Specific growth rate (SGR) will be calculated with the formula:

$$SGR = \frac{[In(Wt) - In(Wi)] * 100}{t}$$

- 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 will be multiplied by 100 to express it in percentage per day

Statistical analyses of the data will be done by one-way 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.

Trainings and Deliverables

Item	Mechanism (e.g. podcast, reports, factsheets).
Captive breeding results	Basic nutrition profile of lungfish grow out expressed in a technical report for extension
Captive reproductive results	Journal article
Captive growth results	Basic guidance on management of lungfish expressed in a farmer-oriented leaflet

Schedule

Task	9/16	10/16	1/17	3/17	6/17	12/17	2/18
Collect fingerlings from 4 zones	x	x	x				
Develop captive breeding	x	x	x	x	x	x	x
Assess reproductive performance	x	x	x	x	x	x	x
Assess captive performance (On-farm and station)		x	x	x	x	x	x

APPENDIX: Hormone protocol

Synthetic gonadotropin releasing hormone analogs or GnRHa are effective on ovulation and spermiation and last longer in the fish's system.

Approach

Determining the hormone dose;
1. Weight of fish: Brood fish will be weighed under water to stress conditions.
2. Recommended dose: using the formulae below,

$$\text{Hormone Concentration} = \frac{\text{Recommended Dose x Fish Weight}}{\text{Desired Injection Volume}}$$

3. Total weight of dose;

Total Weight of Hormone = Recommended Dose x Total Weight of Fish

4. Ripe broods of lungfish (*Protopterus aethiopicus)* will be selected from the culture systems (tanks/ponds). To induce ovulation, intra-muscular injection of synthetic GnRHa will be done using the dose described by Rottmann et al., (1991). The recommended dose for GnRHa will not exceed 300 *u*g/Kg
5. Broods will be injected twice using calculated volume;

Volume of Injection = Recommended Dosage x Fish Weight Hormone Concentration

- First injected dose will be 40% volume
- Second/resolving dose will be 60% after 18 hours from the time of administering the first dose

TOPIC AREA

QUALITY SEEDSTOCK DEVELOPMENT

❖

GENETIC DIVERSITY OF STRIPED SNAKEHEAD (CHANNA STRIATA) IN CAMBODIA AND VIETNAM

ASIA PROJECT: CAMBODIA & VIETNAM
US Project PI: Robert Pomeroy, University of Connecticut – Avery Point
HC Project PI: So Nam, Inland Fisheries Research and Development Institute

Quality Seedstock Development/Study/16QSD01UC

Collaborating Institutions and Lead Investigators

University of Connecticut-Avery Point (USA)	Robert Pomeroy
Inland Fisheries Research and Development Institute (Cambodia)	So Nam
	Uy Sophorn
	Chheng Phen
Can Tho University (Vietnam)	Duong Thuy Yen
	Tran Thi Thanh Hien
	Pham Minh Duc

Objectives

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 wise recommendations for (1) striped snakehead domestication and selection breeding and farming in Cambodia, and (2) possible exchange of snakehead genetic resources between Cambodia and Vietnam.

Significance

The striped snakehead (*Channa striata*) is one of the most widely distributed snakehead species with a native range covering southern China, Pakistan, most of India, southern Nepal, Bangladesh, Sri Lanka and most of the Southeast Asian countries (Froese and Pauly, 2010). It is well-represented in the ditches, swamps, lakes, paddy fields, irrigation canals, small streams, mining pools, and old ponds, showing the highest preference for muddy stagnant waters. Striped snakehead is often utilized in the biomedical field in many local Asian communities (Mat Jais et al., 1994; Baie and Sheikh, 2000). The striped snakehead is an economically important species in both culture and capture fisheries throughout Southeast Asian countries including Cambodia and Vietnam.

This species is being extensively farmed, particularly in the Lower Mekong River basin of Cambodia and Vietnam. In Cambodia wild snakeheads are generally cultured in smaller cages and ponds. Feed represents more than 70% of the total operational cost and the main type of feed for snakehead culture is small-sized or low value fish, representing 60 to 100% of the total feed used depending on feeding strategies adopted by different farmers (So et al., 2005). During the dry season (October to May), the most important source of feed is freshwater small-sized fish, while more marine small-sized or low-value fish species are used during the rainy season (June to September) (So et al., 2005). Importantly, the snakehead production contributes more than 70% of total aquaculture production in Cambodia and one of the major aquaculture fish species in Mekong Delta of Vietnam, which has been farmed since the 1990s (So Nam, 2009; Sinh et al., 2012) due to its popularity as food being found in most Cambodian and Vietnamese dishes at all wealth class levels (i.e., from poor, medium to rich people) and high market and trade demand.

The government of Cambodia put a ban on snakehead farming in September 2004 by the Announcement No. 4004 kor.sor.ko.sor.chor.nor. The reason for this was the potential negative impacts on wild fish 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 also potential negative effects on poor consumer groups from decreased availability of small-sized fish due to dependency of snakehead aquaculture on small-sized (So et al., 2007). In order to remove this ban, the same Announcement mentioned that successful technologies of domesticated breeding, weaning and rearing/growing-out of snakeheads using formulated diets should be developed and applicable in on-station and on-farm levels in Cambodia. 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. This project will support the development of sustainable snakehead aquaculture in Cambodia through genetic research for snakehead domestication and selective breeding and farming in Cambodia.

During the second phase of AquaFish CRSP (2009-2011), the wild striped snakehead broodstocks were successfully developed, matured and semi-artificially induced spawning using the hormone HCG on-station in Cambodia (So et al., 2011). The striped snakehead aged 30 days old after hatch could gradually and successful accept AquaFish CRSP Snakehead Formulated Feed developed by AquaFish CRSP project (Hien & Bengtson, 2009; 2011) in replacement of small-sized fish in the rate of 10% every three days for a period of 30 days of feeding (So et al., 2011).

Under third research phase funded by AquaFish Innovation Lab, the Inland Fisheries Research and Development Institute (IFReDI) at Cambodia's Fisheries Administration further set out (1) to compare performance of domesticated (Vietnamese) vs. non-domesticated (Cambodian) striped snakehead with regard to weaning performance and grow-out on pellet feed; (2) To assess economic efficiency of experimental grow-out of the two types of snakehead on different diets; and (3) To assess product quality of the two types of striped snakehead. The study concludes that both Vietnam hatchery snakehead (domesticated) and Cambodia wild snakehead (non-domesticated) can accept formulated feed, with similar product quality. However, Viet Nam hatchery snakehead show higher survival rate, growth rate and profit than Cambodia indigenous wild snakehead because Vietnam hatchery snakehead has been undergone domestication and selection breeding for more than 20 years (Nen Phanna et al., 2015). To release the current ban on snakehead farming, which has now been in force for 10 years and achieve sustainable development of snakehead aquaculture in Cambodia, the study recommended that genetic diversity of striped snakehead collected from different natural water bodies should be assessed for further success in conducting domestication breeding, weaning and growing-out programs in Cambodia. Now that the snakehead ban is released and if the wild snakehead fishery is sustainably managed in Cambodia, there is good potential to increase trade and marketing of fresh and processed forms of snakehead in Cambodia as well as with other Mekong riparian countries such as Lao PDR, Thailand and Viet Nam, and other countries in Asia, Europe, America and Australia, and to enhance investment of snakehead aquaculture in Cambodia.

Genetic diversity plays important roles in wild and captive populations, indicating ability of a population to adapt to changing environments and the potential of sustained genetic improvement (Allendorf & Luikart, 2007). In the wild, low genetic diversity can cause risks of endanger or extinction of a population under severe environments (Frankham, 2005). Genetic diversity of wild 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 results 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 the first and important step to establish good base populations.

This study will focus on (1) characterization and comparison of genetic diversity of both Cambodia wild and Vietnamese hatchery striped snakehead collected from each sampling locality inferred from mitochondrial DNA marker; and (2) provision of basic information and wise recommendations for striped snakehead domestication and selection breeding and farming in Cambodia and for possible exchange of genetic resources of this species between Cambodia and Vietnam.

Quantified Anticipated Benefits

This research will provide basic information on genetic diversity of both Cambodia wild and Vietnamese hatchery striped snakehead for supporting domestication breeding, weaning and growing-out in Cambodia in order to inform snakehead culture in Cambodia. The following are quantifiable anticipated benefits:

- At least 20,000 farmers in Cambodia will benefit from this Investigation by restarting their snakehead farming leading to increased household income and improved snakehead fish market and trade.
- 250 scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staff, and private sector working on the issues of snakehead aquaculture in Cambodia and Vietnam as well as in other Mekong riparian countries will be better informed about research methods and findings, and have better recommended policies and strategies for sustainable snakehead aquaculture in the region.
- Two (under)graduate students will be supported and trained by this investigation through their BSc/MSc thesis research.
- At least 1,000,000 indirect beneficiaries in Cambodia and Vietnam as well as other Mekong riparian countries who consume snakehead fish in their protein diets leading to improved household food security and nutrition.
- Benefits to the US include improved information and knowledge on population genetic diversity of snakehead for domestication and sustainable aquaculture in Cambodia and Vietnam and this aquaculture is considered as a climate change adaptation measure.

Research Design and Activity Plan

Location

Fish samples of the striped snakehead (i.e., fin clips) will be collected in both Cambodia from the wild and Vietnam from hatcheries where snakehead breeders are also collected for condition and domestication at the hatchery of Freshwater Aquaculture Research Center (FARDeC), Cambodia (See Figure 1). DNA lab work and analysis of Cambodia wild (non-domesticated) snakehead populations will be conducted at Inland Fisheries Research and Development Institute (IFReDI), Cambodia. Similar work of Vietnam wild and cultured snakehead populations will be conducted at College of Aquaculture and Fishereis, Can Tho University (CTU), Vietnam.

Two on-the-job/site trainings on (1) basic fish population genetics and (2) DNA lab work, genetic data analysis, and reporting provided by Can Tho University (CTU) members will be conducted at CTU and at IFReDI, respectively. Genetic data generation, analysis and reporting will be jointly carried out by IFReDI and CTU researchers at IFReDI.

Methods

Fish sampling: Sampling locations are designed based on So Nam and Sam Narith (2011) and key criteria including geographic distance, minimal human disturbance and high abundance of wild snakehead populations, and the popularity and scale of hatchery snakehead stocks/populations (Figure 1). In Cambodia, wild (non-domesticated) snakehead samples will be collected from five provinces in floodplains of Tonle Sap Lake: Battambang (BB), Siem Reap

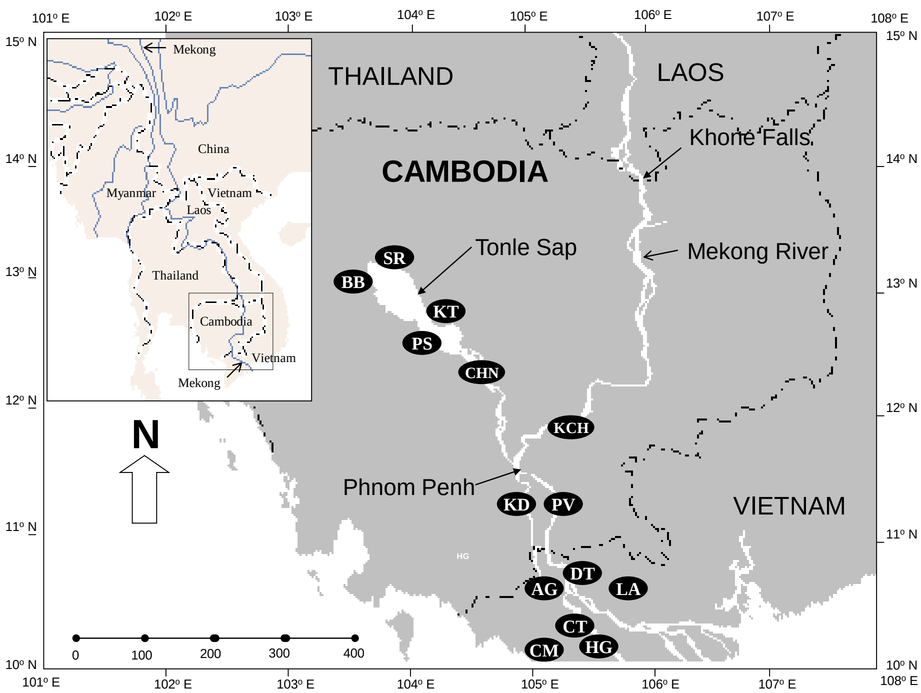


Figure 1: Location map showing sampling locations of the striped snakehead *Channa striata* in Cambodia and Vietnam. BB: Battambang province, SR: Siem Reap province, PS: Pursat province, KT: Kampong Thom province, CHN: Kampong Chnang province, KCM: Kampong Cham province, KD: Kandal province, PV: Prey Veng province, AG: An Giang province, DT: Dong Thap province, CT: Can Tho province, HG: Hau Giang, CM: Camau, and LA: Longan province. Source: Modified from So et al., (2006).

Fin clips from (SR), Pursat (PS), Kampong Thom (KT), and Kampong Chnang (CHN) provinces were collected for the study by So Nam and Sam Narith (2011) and stored at room temperature for the past six years causing the samples to degrade and become unviable for this study. In addition, fin clips will be also collected from other three provinces in floodplains of the upper and lower Mekong River (Kampong Cham, KCH and Prey Veng, PV, provinces) and Bassac River (Kandal, KD, province) in order to have all different populations of the striped snakehead from all geographical areas of Cambodia. In Vietnam, hatchery (domesticated) snakehead samples will be collected from three hatcheries in Dong Thap, An Giang and Can Tho provinces, where most snakehead fingerlings are produced and supplied for other provinces in the Mekong Delta, Vietnam. In addition, wild fish will be collected at three populations from Lang Sen conservation park (Longan province), U Minh conservation park (Camau province), and rice-fields in Haugiang province. These wild populations are hypothesized to be exposed to different levels of human disturbance and be genetically different. At least 50 fish samples will be collected from each sampling location. Fin clips of all collected individuals will be preserved in 100% ethanol and stored in refrigerator for DNA analyses.

DNA extraction: DNA will be extracted using a commercial kit (e.g. AQUAGENOMIC™kit, MultiTarget Pharmaceuticals, Salt Lake City, Utah 84116) according to the manufacturer's protocol. DNA extracts can be checked for quantity and quality by using agarose gel electrophoresis.

mtDNA marker selection and polymerase chain reaction (PCR) optimization: Cytochrome b gene from mitochondrial DNA (mtDNA) will be selected for PCR amplification. This gene has been used in other genetic studies on genus *Channa* (Abol-Munafi et al., 2007; Adamson et al., 2010) and other fish species (Nazia et al., 2010; Sousa-Santos et al., 2014). Cytochrome b exhibits higher levels of genetic divergence within fish species (Abol-Munafi et al., 2007) compared to other mtDNA genes (Adamson et al., 2010), and it is also a powerful marker for phylogeographic studies (Gaither et al., 2011; Hulsey & García-de-León, 2013). PCR conditions will be optimized based on previous studies and PCR products will be visualized on 1.7% agarose gels stained with ethidium bromide and purified (QIAGEN Sciences, Maryland 20874, USA) according to the manufacturer's instruction. Purified products will be sent for DNA sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia).

Data analysis: Multiple cytochrome b sequences will be aligned and all unambiguous operational taxa units will be compiled for editing using ClustalW implemented in MEGA v. 6.0 (Tamura et al., 2013). DNA sequences will be translated into protein to ensure accurate alignment and detection of nuclear mitochondrial DNA (numt), if present. The aligned sequences will be exported to Collapse v. 1.2 (Posada, 2004) to construct a haplotype datasheet. Three estimates of genetic diversity measurement to describe DNA polymorphism at each sampling location will be calculated using Arlequin (Excoffier & Lischer, 2010). The first, haplotype/gene diversity (Hd), measures the probability of uniqueness of a haplotype in a given population. The second, nucleotide diversity (p), is the mean number of pairwise nucleotide differences among individuals in a sample. The third, theta S (qs) (Watterson, 1975), is a measure of the number of segregating sites among haplotypes in a sample. Phylogeographic structure of wild snakehead populations (in Cambodia and Vietnam) will be analyzed using several programs including MEGA v. 6.0 (Tamura et al., 2013) and NETWORK (Bandelt et al., 1999).

Trainings and Deliverables

The deliverables of this investigation will include (1) final technical report, including policy recommendations, and (2) factsheet.

Short-term training

Two short-term trainings will be carried out to improve capacity of IFReDI staff members on (1) basic fish population genetics and (2) practical skills on DNA lab work, genetic data analysis, and reporting.

Long-term training

Two graduate (MSc) students will be partially supported for their thesis work under this investigation.

Schedule

The duration of implementation of this proposed investigation will be 24 months, starting from 1 March 2016 till 28 February 2018.

Activity	Beginning	Ending
Collection of fish samples from the wild in Cambodia	March 2016	April 2016
Collection of fish samples from hatcheries in Viet Nam	March-2016	April-2016
Two-week Training on "Basic fish population genetics at Can Tho University, Viet Nam (provided by CTU)	April-2016	April-2016
Set up DNA lab at IFReDI and hand-on training on DNA analysis (provided by CTU)	May 2016	June 2016
DNA lab work and analyses	July 2016	September 2017
Data analyses and reporting (IFReDI and CTU team members jointly work together at IFReDI)	October 2017	December 2017
Consultation and dissemination workshop on research findings	January 2018	February 2018
Finalization of final technical report	January 2018	February 2018

IMPROVING SEED PRODUCTION OF SAHAR (*TOR PUTITORA*) IN CHITWAN NEPAL

ASIA PROJECT: NEPAL
US Project PI: James S. Diana, University of Michigan
HC Project PI: Madhav Shrestha, Agriculture and Forestry University

Quality Seedstock Development/Study/16QSD02UM

Collaborating Institutions and Lead Investigators

University of Michigan (USA)	James S. Diana
Agriculture and Forestry University (Nepal)	Jay Dev Bista
	SK Wagle
	Madhav K. Shrestha

Objectives

1. To continue development of sahar breeding technology in Chitwan.
2. To improve protocols for manual detection of maturity and manual spawning of sahar in Chitwan.
3. To determine if gonadotropic hormone injection can be a feasible method to induce maturation in sahar.
4. To establish nursing and rearing management practices of sahar fry in Chitwan.

Significance

Sahar (*Tor putitora*) is an economically important, high-value indigenous fish species in Nepal (Rai et al., 2007). The price of sahar in the Nepalese market is almost double the commonly cultivated carp and tilapia species. Sahar is still taken in capture fisheries in lakes and rivers, but no commercial cultivation has begun in Nepal. This species is declining from its natural habitat, mainly due to urbanization, illegal encroachment, over-fishing, and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista et al., 2008). The population status is considered endangered by the International Union for the Conservation of Nature (IUCN 2016) due to over harvest and habitat destruction.

Various national aquaculture plans for Nepal, most recently NARC (2010) and FAO (2013), have included the development of cold water systems for aquaculture in upland areas as a priority. In addition, seed production is also recognized as a major bottleneck to aquaculture development in Nepal. The potential culture systems for cool water areas include development of trout culture from imported fish and the use of indigenous fish like sahar for aquaculture. Rainbow trout culture is expanding in the country with government assistance, and all components of the culture business are developing. Culture of indigenous species is a high priority globally, as it reduces issues with invasive species introductions, is in harmony with local cultural needs, and increases the possible options for aquaculture production. However, for many indigenous species, particularly sahar, production of seed is very limited and is currently allocated mainly for restocking natural waters. This proposal seeks to produce large volumes of sahar seed and develop nursing and rearing techniques, so sahar culture can be extended from experimental farms to more commercial systems and help meet the need for additional restocking of natural waters. The latter target has clear impacts on environmental sustainability, while even commercial production for food would reduce pressure on wild stocks and enhance their survival.

Attempts to culture and conserve this species were initiated in Nepal with major efforts to develop culture technology and propagate the species (Gurung et al., 2001; Joshi et al., 2002). This has led to a better knowledge of spawning biology, ecology, and behavior of sahar, as well as preliminary growth performance in captive conditions. Enhanced growth in tropical and subtropical ponds and the recent breeding success in hatcheries have raised new hopes on the prospects of sahar aquaculture in Nepal

(Shrestha et al., 2005, 2007; 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, helping to halt the decline of this native species. Due to its omnivorous and predatory feeding, sahar has also proved to be a good candidate to co-culture with mixed-sex tilapia to control tilapia recruits in a pond 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 (Jaiswal, 2012).

Sahar is known to be intermittent in spawning behavior. Details from the propagation experiences in Pokhara indicate that it can spawn in most months, except January, but in natural waters, it spawns during the monsoon when rivers and streams are at peak flow. Sahar typically migrate a long distance from large rivers to streams for spawning. The Fisheries Research Center in Pokhara is the only location where fry are produced, and this production is still in limited quantity. Demand for sahar fry has increased for restocking in rivers and lakes and for aquaculture production. Lack of availability of fish seed is a major bottleneck for commercial production and conservation. Sahar breeding has been attempted in the Aquaculture and Fisheries Department of AFU in Rampur, Chitwan, and was successful in producing 250 fry during a practical class in 2010.

We conducted an experiment at the Department of Aquaculture and Fisheries, Agriculture and Forestry University, Rampur, Chitwan during the last experimental cycle of the AquaFish project to explore and assess breeding performance of sahar in the Terai region, which has a subtropical climate. Twenty-eight male (0.5-1.5 kg) and 35 female (0.8-2.5 kg) brood fish were reared in ponds at 1,000 kg/ha and provided 35% protein feed at 3% body weight per day. Maturity was observed by sampling fish and applying pressure to the abdomen to express gonads every two weeks during off season; the frequency was increased to every third day as breeding season approached. One female sahar (3-5 years old) was ready for breeding in March when water temperature was 23.3-25.2 °C. Males about 1-2 years old expressed milt in almost all months. Ova from mature females were obtained by simple hand stripping and fertilized with milt manually collected from males. The fertilized eggs were incubated in Atkin hatching trays. Survival and growth of fry were high, and maturation details were similar to fish spawned under temperate conditions. This study demonstrated that natural breeding and fry rearing is possible in the Terai region of Nepal.

In addition to natural spawning, attempts were made to induce three females to mature by injection of ovaprim. At 26 hours after ovaprim injection, some began to release eggs. Milt from two males was used to fertilize these eggs. Fertilized eggs were incubated as before, and a similar process was repeated. Hatching occurred after 96-104 hrs at 23-25 ^{o}C and 80-88 hrs at 25-29 ^{o}C.

While the previous experiments demonstrated that fry production can be successful in a sub-tropical climate, there remains a need for further studies on synchronization of breeding time and mass seed production. Maturity of female fish was monitored biweekly before the breeding season (May-August). Male fish were always found ripe with oozing milt after pressing their belly, but females were not ripe during May-August. In spite of frequent checking on maturity, only 1 out of 35 female fish reared was found at the right time to perform manual spawning, while 15 female fish were found to be over mature during regular observations. This indicates that females ripen very quickly and become overly mature rapidly after that. We need to either increase the frequency of maturity determination to daily during the spawning season, or determine improved ways to use ovaprim to induce maturation of premature fish.

This study is intended to refine the breeding technology, nursing, and fry production developed in the earlier experiment in order to increase availability of sahar fry for culture. While sahar are listed as endangered by IUCN, they are available for consumption and sport fishing in Nepal, and there are no restrictions to using them in aquaculture settings in the country.

Quantified Anticipated Benefits

The results of this study will further improve methods to produce sahar fry for restocking and will provide an additional fry for aquaculture in Nepal. We expect to improve seed production at the new site in Rampur and, as a result, should produce additional fry beyond our previous experiments. We hypothesize that induced maturation will be more successful than manual checking, and will also reduce effort since it will not be necessary to check for maturity on a daily basis. Availability of cultured sahar for restocking should help enhance sahar populations in natural waters. We expect to stock fry in at least five different natural waters as a result of our seed production. For Nepal, we intend to initiate outreach on production of sahar seed by conducting at least one workshop on our improved methods and results. Through this, we intend to train at least ten fish hatchery workers and ten women farmers in the induction technology.

One workshop with ten fish hatchery farmers and ten commercial fish farmers, in addition to government research scientists and extension officers being trained on sahar seed production. One report clarifying sahar production techniques. At least three graduate or undergraduate students trained on sahar reproduction techniques and research methodology.

Research Design and Activity Plan

Location

The Aquaculture Farm of AFU at Rampur and a private farm at the Center for Aquaculture Research and Production (CARP), Kathar, Chitwan, will be sites for testing of improved culture protocols.

Methods

Null Hypothesis. There are no differences in breeding and growth performance of sahar between the two spawning methods.

1.1 At least 30 female and 50 male fish (1-kg size or larger) will be maintained as brood stock in Chitwan.

1.2 Pond Facilities: earthen ponds of 200 m^2 and 4 concrete tanks at 50 m^2 each.

1.3 Culture Period: two years for mature brood maintenance and breeding.

1.4 Nutrient Input: daily feeding with locally made feed containing 35% protein at 2-3% BW.

1.5 Water management: maintain at 1.0-1.5 m deep. Water quality monitoring will be conducted using standard protocols, with monthly water sampling.

1.6 Over 600 sahar should be held for brood stock development in Chitwan ponds. We will monitor oocyte development (using cannulation on a subsample of about ten fish per month), gonadosomatic index (GSI) of brood stock fish that are stripped of eggs at maturation, and egg somatic index (ESI) for these same fish. Temperature and other conditions will be monitored over the brooding period. The most promising brood fish will be transferred to concrete tanks to allow for easier collection and regular determination of maturity.

1.7 Half of the females deemed to be near maturity will be injected with gonadotropic hormone (ovaprim/ovulin) at 5 mg/kg body weight. They will be held separately in small tanks and checked for maturity daily. Females with mature eggs will be used for manual spawning.

1.8 Half of the nearly mature females will continue to be held and checked daily for maturity. Those found with mature eggs will also be used for manual spawning.

1.9 Spawning will be done using the dry method, with eggs removed from females by pressure on the abdomen, then sperm from males using similar methods. Eggs and sperm will be mixed, and the fertilized eggs washed several times before moving them into Atkin incubators. One layer of eggs will be allowed to settle on a single mesh screen in the flow-through system. Water flow will be maintained at 7-9 L/minute. The incubation trays will be covered with a dark cloth to reduce light levels in the tray. The eggs will be observed 24 hours after initial incubation, and unfertilized eggs counted. Dead eggs will be counted and removed each day to protect healthy eggs from fungal infection. After 4 days (96 hours) hatching should occur over a 24-hour period, when distinct eyes will be seen in hatchling fish. After attaining free-swimming stage, the larvae will be transferred into a nursing and feeding tank of 2.5 m x 0.4 m x 0.3 m dimension.

1.10 Fry will be reared in outdoor ponds, first by promoting natural feeds with fertilizer in the ponds, later using supplemental feed after fry have been successfully weaned.

1.11 Growth will be estimated over one year, with monthly sampling of fish size and apparent health for fingerlings raised in ponds.

2. Statistical Design:
Performance of sahar breeding (ESI, GSI, survival of fry, and growth of fry) will be compared between induced and naturally spawned fish.

3. Statistical Analysis: ANOVA or T-test as appropriate.

Trainings and Deliverables

Training: Ten government hatchery workers will be trained in a workshop on our new induction technology for sahar.
Ten private fish farmers will also be trained through a workshop on sahar breeding technology.
At least three graduate students will be trained on reproductive technology for seed production in aquaculture through involvement in this project.

Deliverables: One workshop on sahar induction technology.
One final report and hopefully one publication after the grant period on this technology.
One fact sheet on sahar production technology.

Schedule

Brood stock collection and maintenance will begin 1 May 2016; fry rearing is planned to occur from November 2016 through December 2017 (2 years of spawning seasons). Final report will be completed no later than 28 February 2018.

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

ASIA PROJECT: BANGLADESH
US Project PI: Russell Borski, North Carolina State University
HC Project PI: Shahroz Mahean Haque, Bangladesh Agricultural University

Human Nutrition and Human Health Impacts of Aquaculture/Experiment/16HHI01NC

Collaborating Institutions and Lead Investigators

Khulna University (Bangladesh)	Khandaker Anisul Huq
Bangladesh Agricultural University (Bangladesh)	Md. Ashraful Islam
	Sadika Haque
	Shahroz Mahean Haque
Shushilan NGO (Bangladesh)	Sattyananda Biswas
North Carolina State University (USA)	Russell Borski

Objectives

1. Evaluate the effects of different fertilizers on production of prawn, *Mola* and carps in gher/pond polyculture systems.
2. To 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.

Significance

Wide 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). In Bangladesh, 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. The present investigation proposes to address this problem by promoting the use of the *Mola* (*Amblypharyngodon mola*) fish and seasonal vegetables in integrated aquaculture-agricultural systems and by evaluating the impact of different fertilizers on the production of these nutrient-rich foods as well as prawn and carps.

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 leading to night blindness and up to 55% exhibit 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 concentration of vitamin A (~1900 IU, Thilsted et al., 1997) and micronutrient content than other commonly consumed fishes (e.g., carp). The *Mola*, a small fish with soft bones, is particularly favored in the diets of many people; however, consumption in the Southwest region 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. Additionally, their bacteria-enriched waste can be utilized to enhance prawn production. Similarly, carps also feed on natural pond productivity and hence can be grown without supplemental feeds. Moreover, combining species of different trophic levels can maximize nutrient utilization and decrease the potential for harmful phytoplankton blooms and poor water quality that leads to mortalities (Halver, 1984; Wahab et al., 2008).

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 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 two 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 (30kg) and yeast (400g/ha) was 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 experiment will evaluate 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). Hence, 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.

Quantified Anticipated Benefits

1. We will determine whether organic (yeast + molasses), inorganic, or a combination of the fertilizers provide better production yields and economic returns for farming *Mola*-prawn-carp in gher/pond polyculture systems.
2. Proper selection and more efficient approaches to vegetable production using pond muds, mulch or fertilizer will be established.
3. Better management practices for cultivating nutrient-rich *Mola* and vegetables for family consumption will increase the dietary nutrition available for low-income farming households, especially for women and children.
4. Forty to sixty women and girls will receive direct, on-site instruction on nutritional benefits of integrated farming designs and on best-management practices for production of nutrient rich fish and vegetables for consumption.

Research Design and Activity Plan

Location

This investigation consists of a series of two studies and training activities, which will be carried out on participating farms located in villages of Rangpur Union, Dumuria Upazila, Khulna District, Bangladesh and the surrounding region. Water quality, and both pond dyke soil and the pond mud of the proposed experiment will be analyzed at Khulna University and BAU, Mymensingh, Bangladesh, respectively.

Methods

Experiment 1. Evaluate the effects different fertilizers on production of prawn- Mola- carps in gher-pond polyculture farming systems.

Null Hypothesis 1: There is no difference in fish/shellfish yield or benefits of applying organic and inorganic or their combination on Mola-carp-prawn gher-pond polyculture.

Most of the farming households practicing traditional prawn culture in gher systems minimize the use supplementary feeds for prawn/fish culture because of high investment costs (50-60% of the total cost), hence, application of the fertilizers is a critical component to increasing production of prawns and fishes. Here we will assess 3 different fertilizer applications in prawn-*Mola*-carp polyculture on the farms of 20 households such that each treatment group will be replicated 5 times. One treatment will apply molasses (30 kg/ha) and powdered yeast (0.4 kg/ha) that was used in our previous work demonstrating *Mola* and carps could be successfully integrated into prawn culture. A second will incorporate inorganic fertilizers using levels recommend for extensive or semi-intensive culture of fishes (4N:1P; 28 kg N/ha using Urea; 7 kg P/ha using triple super phosphate; see Egna and Boyd, 1997), and a third will incorporate both types of fertilizers at 50% of the application amount used in the other groups. A fourth treatment will have no fertilizer applied as a reference group. The design is as follows:

Parameter	T1	T2	T3	T4
Prawn (*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
Fertilization application	0	Molasses + Yeast	Inorganic Fertilizer	50 % of T2 + 50% of T3
Replication	5	5	5	5

Nursery pond preparation for prawn will be done according to standard practices followed by farmers. Prior to gher drying, bottom mud will be excavated and ponds will be limed and filled. Juvenile prawn (*Macrobrachium rosenbergii*) will be stocked at a density of $2/m^2$, Rohu at $0.1/m^2$, and brood *Mola* at $2/m^2$. Fertilizers will be applied to ponds (average of ~50 decimal or 2000 m^2; 1.0-1.5 m depth) fortnightly. Prawn will be fed with a commercial feed using a feeding tray at 10% body weight (bw)/day for 10 days, 7% bw/day for 10 days, 5% bw/day for 10 days and 4-2% bw/day for rest of the culture period. Feeding frequency will be twice daily in the early morning and evening. Fish and prawn will be grown out for six months. After 60 - 70 days *Mola* begin to self-recruit and hence can be harvested for home consumption. Partial harvesting of larger *Mola* will be encouraged beginning at day 75 and periodically throughout the remainder of the experiment. Number and weights of partially harvested *Mola* will be recorded.

Water quality parameters, including temperature, secchi disc (transparency), DO, and pH will be measured on the spot weekly, and total alkalinity, nitrate-nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N), and phosphate-p (PO_4-P) will be measured fortnightly.

During the six-month grow-out period, performance data (weights/lengths) for all species will be collected by monthly sub-sampling, followed by total yield assessment at the end of experiment. Significant differences ($p < 0.05$) in water quality, total production yields (biomass), and prawn, *Mola* and carp production yields/growth will be determined by analysis of variance (ANOVA) using SPSS.

Experiment 2. To 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.
Null Hypothesis 2: There is no difference in yield or benefits of summer or winter vegetable production between the different pond muds from the fish polyculture treatments or different soil treatments.

Two activities will be conducted under this trial, one on summer and the other on winter vegetable cultivation on pond dykes. Pond muds derived from the different polyculture gher pond systems in Experiment 1 (T2-T4) will be evaluated for their effects on production of fruit vegetable crops over both winter (Okra or gourd) and summer (tomato) seasons. This will allow a determination on whether vegetable growth might be enhanced by a particular pond fertilization treatment (organic versus inorganic fertilizers or both combined) using both summer and winter vegetables. The effects of pond mud will be compared in the absence and presence of mulch (black polythene) and with mulch and inorganic fertilizer. Black polythene is generally effective in preserving soil moisture and it will help to reduce the cost of irrigation, labor cost of weeding as well as to reduce the effect of soil salinity in experimental plots and can be incorporated at relatively little cost. The third group will have additional application of inorganic fertilizer at half the recommended level used for crop production in soils whether on pond dykes or not (FRG, 2012). Use of inorganic fertilizer represents a growing practice of horticulture on Bangladesh homesteads but the treatment can represent a significant cost, so here we will evaluate if its addition provides any further benefits relative to its extra cost. The experimental design is as follows:

Vegetable	Pond Treatment Experiment **1**	Soil Treatment 1	Soil Treatment 2	Soil Treatment 3
Summer Fruit: Okra or gourd	Pond T1	Pond mud	Pond mud + mulch	Pond Mud + mulch + inorganic fertilizer
	Pond T2	Pond mud	Pond mud + mulch	Pond Mud + mulch + inorganic fertilizer
	Pond T3	Pond mud	Pond mud + mulch	Pond Mud + mulch + inorganic fertilizer
Winter Fruit: Tomato	Pond T1	Pond mud	Pond mud + mulch	Pond Mud + mulch + inorganic fertilizer
	Pond T2	Pond mud	Pond mud + mulch	Pond Mud + mulch + inorganic fertilizer
	Pond T3	Pond mud	Pond mud + mulch	Pond Mud + mulch + inorganic fertilizer
Replicate Plots	3	3	3	3

Three replications will be done for each of the summer and winter activities, where total number of plots will be 3 replicates x 3 pond treatments x 3 soil treatments = 18. Plot size of the dyke will be 2 m x 2 m. The inorganic fertilizer will be applied according to the fertilizer recommendation guide (FRG, 2012).

Plant growth (plant height, leaf number, branching number) and flowering time, fruit yield parameters (tomato, gourd or okra), yield per plot and converted to per hectare and some nutrient quality parameters of vegetables will be collected in each season. Vegetables consumed by household will be recorded. A marginal cost-benefit analysis evaluating return on investment among the three soil treatments will be done based on total production yield, input costs and value of crops. Differences among variables measured will be analyzed by 3-way ANOVA in SSPS.

A major element of this investigation is to empower women and girls on best practices for producing crops, namely fishes and vegetables, for household consumption or sale that require relatively little additional cash investment and that can enhance nutrition and income. It is well established that a key role for women and girls in household farming is to maintain fish or seafood crops and establish homestead gardens (Belton et al., 2011). Gardens on pond dykes are less susceptible to flooding and muds provide a good source of nutrients for vegetable production at minimal costs. Thus, the technologies developed from our previous project and here for *Mola* and dyke cropping our excellent opportunities to work with women to increase household consumption of nutritious foods. We will work directly with women and girls in participating households in undertaking the studies. They will be trained by the PIs on all aspects of the experiment: the purpose and goals of the work, methodology, tracking inputs, cost accounting, consumption, marketing opportunities etc. They will be provided record keeping books to monitor feeding and fertilization inputs, their costs and sales, and consumption. This will be complemented by training sessions on nutrition, *e.g.* caloric intake, importance of balanced diets, and value of *Mola* and vegetables to the diet. Their food consumption will be monitored through a simple survey prior to and after the studies and relative to that of reference non-participating households (60 women and girl members total). Collectively the work will provide critical training on nutrition and best management practices for producing household foods in integrated AAS that can enhance the nutrition of women and children including an estimated 60 individuals from participating and non-participating households.

<u>Trainings and Deliverables</u>

1. The findings from these experiments will be reported through the Technical Reports of the AquaFish Innovation Lab (FIR).
2. The results will also be reported in workshops (Investigation 5) and/or relevant conference presentations.

3. Approximately 60 women and girls will receive direct training on nutrition and best management practices for producing fish and vegetables for home consumption that can benefits to their wellbeing.
4. The management practices for improved gher-prawn/*Mola*/carp or gher-pond/dyke cropping found in the proposed studies will be disseminated to the wider farming community through workshops outlined in Investigation 5.

Schedule

June 2016 to March 2017: Experiment 1:
September 2016 to September 2017: Experiment 2
June 2016 to December 2017: Training
January 2018 to February 2018: Final Investigation Report

FISH CONSUMPTION AND IMPLICATIONS FOR HOUSEHOLD NUTRITION AND FOOD SECURITY IN TANZANIA AND GHANA

AFRICA PROJECT: GHANA & TANZANIA
US Project PI: Kwamena Quagrainie, Purdue University
HC Project PI: Steve Amisah, Kwame Nkrumah University of Science & Technology

Human Nutrition and Human Health Impacts of Aquaculture/Study/16HHI02PU

Collaborating Institutions and Lead Investigators

Purdue University (USA)	Kwamena K. Quagrainie
Sokoine University of Agriculture (Tanzania)	Sebastian Chenyambuga
Kwame Nkrumah University of Science and Technology (Ghana)	Steve Amisah

Objectives

1. Measure household food security in terms of dietary diversity of households using indicator measures.
2. Analyze the determinants of household consumption practices of various food types that includes fish.
3. Formulate policy measures to improve aquaculture and fisheries practices and fish consumption to improve household food security.

Significance

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*" (FAO, 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.

Fish and fishery products contribute significantly towards global food security and human nutritional needs in developing and developed countries. 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 world per capita fish consumption has increased from an average of 9.9 kg in the 1960s to about 19.2 kg in 2012 (FAO, 2014). The global growth in fish consumption has occurred mainly in Asia and the developed world and not in sub-Saharan Africa. 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). The FAO reports that per capita fish consumption either remained static or decreased in some sub-Saharan African countries such as the Congo, Gabon, Liberia, Malawi, and South Africa (FAO, 2014).

Many households in sub-Saharan Africa, especially those in rural settings are prone to food insecurity because of widespread poverty. The issue of food insecurity is heightened by the unstable and seasonal trends in domestic food and fish production. This results mainly from the scale of production; majority of farmers are small scale farmers and fishermen. Several developmental interventions related to fish consumption, aquaculture and capture fisheries have aimed at improving nutritional status of households through direct influence on dietary intake, from food production and an 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. This study focuses on nutritional impact through direct fish consumption and the income effect. Several factors are critical to the nutritional decisions of household. Some factors are 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 depicts the per capita purchasing power and food availability.

There are differences within countries in terms of quantity and variety of fish consumed and the subsequent contribution to food security. Dey et al., (2005) reported that though fish was a major source of protein for low income households in Asia, these households consumed less fish compared to rich households. In Bangladesh, poor households sold the fish from their ponds for cash rather than consuming it at home (Bene, 2003). Thilsted et al., (2014) reported that, in Bangladesh, even though fish is quite expensive, consumption in small quantities made a significant difference in contributing to the nutritional quality of the diets of poor people. In the central region of Malawi, a study of fish farming and non-fish farming households over a period of four weeks revealed no significant differences between the households in terms of their nutritional status because fish farming households cultured the fish mainly for selling and not for consumption (Thilsted et al., 2014). Karim (2006) also reported that, in Lake Chad, households that were plagued by chronic food shortages sold their fish from ponds compared to rich households (Karim, 2006). Studies by Abebaw et al., (2011) and Hailu (2012) have also reported that the level and intensity of food insecurity in developing countries is high and influenced by household socioeconomic factors and poor functioning of marketing systems. In Ethiopia, socioeconomic factors like age, location (whether urban or rural), marital status, educational level, religion, and employment status were found to be strong determinants of household food security (Teller and Yimar, 2000). Clearly, there are differences in household preferences and consumption of fish, which may depend on the availability, cost, disposable income, and other socio-economic and cultural factors.

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 accounts for about 30% of all animal protein, and is a major source of other important nutrients in many households in the Tanzania (FAO, 2007). Notwithstanding the importance of fish to Tanzanian households, growth in fish production has been very low since the early 1990s. Between 1990 and 2010 for instance, fish production increased by an average of only about 0.35% per annum; and per capita production declined by average of about 2.5% per annum, due to rising population. The poor growth in fish production had been attributed mainly to the dwindling trend in inland catch, the main source of fish in the country and an under developed aquaculture subsector. Fish is consumed more among subsistence groups and other low income households, accounting for about 25.7% of food expenditures (Essuman, 1992; FAO, 2004).

In Ghana, fish consumption is higher in coastal, riverine, and inland water areas. A greater percentage of fish consumed comes from marine sources and consumers prefer fresh fish especially those residing along the coast. However, majority of the fish available on the market is in processed forms such as smoked, fried, salted and dried to reduce post-harvest loses. Antwi (2006) reported that about 70% to 80% of all domestically produced marine and freshwater fish is consumed in the smoked form. Catfish and some small palegic species are almost exclusively consumed in the smoked state in most parts of Ghana.

This study examines household food security improvements through household dietary diversity in Ghana and Tanzania. The study examines food security using demand models and a household food security indicator. This is necessary because of the diverse and complex nature of food security (Cunningham, 2005).

Quantified Anticipated Benefits

1. Numerical measures of dietary diversity (proxy for food security) and classification of households into "food secure," "borderline," and food insecure" groups.
2. Identification of the determinants of household food security outcomes and factors that impact aquaculture and fisheries practices.
3. Identification of the factors that determine household consumption of various food types that includes fish.
4. Results will inform important policy and funding decisions and implications on fish consumption and food security in sub-Saharan Africa.
5. Information on how increased fish consumption through increased supply from aquaculture development and sustainable fisheries would improve food security of households through dietary diversity.

Research Design and Activity Plan

The World Food Program (WFP) among other organizations have over the years developed and validated some indicators for assessing food security. These include Coping Strategies Index (CSI), Reduced Coping Strategies Index (rCSI), Household Food Insecurity and Access Scale (HFIAS), The Household Hunger Scale (HHS), Food Consumption Score (FCS), Household Dietary Diversity Scale (HDDS) and a self-assessed measure of food security (SAFS) (Maxwell et al., 2003).

This study will adopt the FCS to assess food security in terms of fish consumption and food security. The FCS was developed by the WFP in response to problems concerning data analysis and reproduction of data. FCS involves the collection of information of food consumed by households and they are weighted differently 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. FCS uses the number of food items eaten and the frequency with which these foods are eaten over a period as a measure of how food secure the household is (Maxwell et al., 2003). FCS ranges over a scale of 0.5 to 112. The main advantage of FCS is that the weights are country specific making it highly correlated with other food security indicators particularly those measuring energy content as well as making it ideal for identifying the dietary pattern of specific foods (Kennedy et al., 2010). However, the indicator can mask differences in dietary patterns (WFP, 2008).

The data for the analysis will be a combination of survey data collected in Ghana and Tanzania under the 2013-2015 AquaFish Innovation Lab funding cycle and national survey datasets from both countries. The 2013-2015 AquaFish Innovation Lab funded dataset includes 126 households in Ghana and 55 in Tanzania in fish farming regions. The dataset provides information on some demographics, cultural, health, and lifestyle factors that reflect farming practices as well as seafood consumption patterns. The additional dataset includes the 2012/2013 Ghana Living Standards Survey (GLSS) data published in August 2014 and the 2011/12 Tanzania Household Budget Survey (THBS) data published in July 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 a total of 16,772 households from all ten regions of Ghana. The combination of the two datasets will ensure that the analysis is constructive, robust, and comprehensive.

The THBS collected information on a range of household factors across Tanzania, which included demographics; economic activities; health factors; sanitation and utility services; household income; ownership of consumer goods and assets; housing structure and materials; and distance to services and facilities. Information is available on food items consumed that includes cereals and grains, animal products (including both fish, meat and other seafood), milk and dairy products, pulses (all legumes), vegetables fruits, fats and oils, and beverages (including alcohol). The information was collected using four main household questionnaires that recorded daily household purchase, consumption and expenditures for 28 days. There is information on a total of 10,186 households from all regions of Tanzania Mainland.

In assessing the impact of fish consumption and household food security outcomes, the main challenge is selectivity bias. This is because households that consume fish are self-selective, which poses a potential bias where the consumption decision is likely to be correlated with the error term in a regression analysis. The endogenous nature of the consumption decision will be addressed in the analysis.

Trainings and Deliverables

Policy recommendations for government and development agencies regarding measures to improve aquaculture and fisheries practices and fish consumption to enhance food security.

Beneficiaries

Policy makers, development agencies, academic researchers, scientific community, etc.

Schedule

Renew / Amend contracts and subcontracts with SUA	August – October 2016
Collation of data and information from the database on food consumption.	November 2016 – March 2017
Data Analysis	March 2017 – December 2017
Reporting	January – February 2018

OUTREACH TO INCREASE EFFICIENCY OF AQUACULTURE IN NEPAL

ASIA PROJECT: NEPAL
US Project PI: James S. Diana, University of Michigan
HC Project PI: Madhav K. Shrestha, Agriculture and Forestry University

Human Nutrition and Human Health Impacts of Aquaculture/Activity/16HHI03UM

Collaborating Institutions and Lead Investigators

University of Michigan (USA)	James S. Diana
Agriculture and Forestry University (Nepal)	DK Jha
	Nabin Khanal
	Narayan Pandit
	Madhav K. Shrestha

Objectives

1. To expand outreach on school ponds in villages, including provision of water quality testing kits for schools, expansion of school ponds to at least two additional communities, and outreach on 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.

Significance

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).

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).

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 level. 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.

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., 2016a). While many Nepalese attend school, most have only a primary school education, and about 68% of women are illiterate. Therefore, training must take these limitations into account, while still providing for information exchange (Kloeblen, 2011). Schools remain the center for learning in a community. Having ponds in the schools produced 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 consider the results of our school pond project a great success. 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. In fact, the ponds were so popular, some neighboring farmers constructed similar ponds within a few months of our school pond construction, and the local people wanted advice and materials to construct a community pond on school property. Pre- and post-training evaluation demonstrated that there was a significant increase in knowledge of the students about aquaculture, with a median grade of <40% on the pre-test and of 61-80% on the post-test. Initial knowledge about the nutritive value and production system of fish was very poor, but by the end of the training, the knowledge of students on fish production and nutritive value of fish was significantly increased.

The school pond project, in addition to our earlier project on household ponds in rural communities, have both led to dramatically increasing interest in initiating aquaculture by local people, as well as expanding the program to other schools. We believe a project to continue outreach on school and household ponds and to advise local communities on aquaculture would continue to spread this knowledge and increase food production to poor rural communities. One objective of this proposal is to continue outreach on aquaculture to our target communities to further enhance our previous educational activities.

Since various outreach activities have been a major component of our work in Nepal, we believe it is time to evaluate the relative success of these activities. For this component, we intend to focus on locations where people have received training or on-farm experiments to improve local fish culture. These locations include the school pond communities, locations where we have conducted periphyton enhancement projects, and locations where we have helped in construction of household ponds. For these areas, we intend to conduct 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. Hence, the third objective of this project is to complete a survey to determine recent changes in fish culture practices and sources of information the led to these changes in several of our target communities.

<u>Quantified Anticipated Benefits</u>

We anticipate that at least 2 new school ponds will be built, 40 more students will be educated on the methods of fish farming, and 20 more women will receive training in fish farming and its role in household health. Finally, we believe our survey results will help identify successes in our fish culture experiments and help coordinate future outreach activities in Nepal.

Training of 40 students and 20 adult women through school pond programs at 2 schools. Added training on research techniques for at least five university students in Nepal.

Research Design and Activity Plan

Location

Public schools will be selected from schools requesting to add ponds to their facilities, most likely in the same areas as our previous pond program.

Methods

1 *School Ponds*:

1.1 A 200 m^2 pond will be establishment for two schools.

1.2 Carps and tilapia will be stocked in each pond and the materials necessary to grow them will be provided to each school system.

1.3 School students and teachers will receive regular training about pond construction and farming activities.

1.4 Focal educational activities include: fish pond design; fish farming, including feeding, fertilizing, growing and handling fish; and nutrition education, including fish cooking and eating.

1.5 Each school in the program (including the four already possessing fish ponds) will receive basic water quality testing kits for incorporation in their teaching. Kits will include simple HACH tests for dissolved oxygen, alkalinity, and pH as well as Secchi disks and thermometers. Sufficient chemical supplies will be provided to continue service of these kits for at least 4 years.

1.6 Informal education activities include forming a women's fish farming group.

1.7 Two meetings and discussions on fish farming will be held with groups of women established at each new school.

1.8 Topics on fish farming will be extended, including managing pond depth, pond preparation, species choice, water color, fertilizing, feeding, growing, and harvesting of fish.

1.9 Topics on health and nutrition will also be extended, including fish preparation and the value of regular consumption.

2. *Outreach Survey:*

2.1 Four locations will be selected where various aquaculture enhancement programs have been conducted.

2.2 A survey instrument will be developed and submitted for IRB approval.

2.3 Surveys will be conducted at 50 households with ponds in each location.

2.4 Statistical Design, Null Hypothesis, Statistical Analysis: no improvements will have been implemented in pond culture among communities. Improvements identified will not be a component of material extended in outreach projects to the communities. Statistical analysis: Chi square.

Trainings and Deliverables

Training: About 40 secondary school students will learn about aquaculture through our curriculum and school pond program.
Approximately 20 women will learn about aquaculture and its role in household nutrition through our new women's groups at the same 2 schools.
Five undergraduate or graduate students will learn about extension and training through involvement in this project.

Deliverables: New school ponds at two locations yet to be determined.
A survey questionnaire about success of various aquaculture extension done through AquaFish in Nepal.
One fact sheet on school ponds.

Schedule

Establish ponds and women's groups: 1 October through 1 December 2016. Class use of ponds: 1 September 201 through 1 June 2017. Survey data collection: 1 April 2017 through 1 December 2017. Final report will be completed no later than 28 February 2018.

WOMEN IN UGANDA AQUACULTURE: NUTRITION, TRAINING, AND ADVANCEMENT

AFRICA PROJECT: KENYA & UGANDA
US Project PI: Joseph J. Molnar, Auburn University
HC Project PI: John Walakira, National Fisheries Resources Research Institute

Human Nutrition and Human Health Impacts of Aquaculture/Activity/16HHI04AU

COLLABORATING INSTITUTIONS AND LEAD INVESTIGATORS

Auburn University (USA)	Joseph J. Molnar Claude Boyd
National Fisheries Resources Research Institute (Uganda)	Gertrude Atukunda Moureen Matuha
Makerere University (Uganda)	Theodora Hyuha John Walakira
Fisheries Training Institute (Uganda)	Gertrude Abalo

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.

Significance

This activity outlines a capstone series of events that will engage 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 builds 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 who can amplify and refract the scientific information about fish culture produced by the project.

Lungfish is an indigenous species in that has reproduced in activity under the aegis of previous AquaFish work led by John Walakira. The work described here endeavors to advance the role of lungfish and other fish species as food items in Ugandan diets. We seek 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 understandings 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 (IFPRI, 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 (2014) 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, 2014).

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 often is 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).

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.

Some women-operated kiosks feature lungfish products (Walakira et al., 2012). 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. Walakira et al., (2012) reported 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.

Quantified Anticipated Benefits

- A Conference on Advancing Nutrition and the Status of Women through Uganda Aquaculture will involve at least 30 participants from women's farming groups, cooperatives, women-led fish-related business, and others in the value chains of tilapia, lungfish, and clarius.
- A training on women's organizations in development will target the leadership of cooperatives and women's organizations.
- Training on value chains, mobile applications, and marketing in aquaculture, although addressed to a broader audience, this training will feature women's involvement in the value chain and women as primary providers of child nutrition in Uganda.
- The Annual Fish Farming Conference and Trade Show is an annual activity that this project will reinforce and infuse with gender-related activities to engage all participants in the ways that women can advance aquaculture in Uganda.
- Three presentations to fish farmer cooperatives will address technical needs and issues, as well as specific gender-related concerns in the operation of farmer associations.

Research Design and Activity Plan

Activity 1: Organize and conduct a conference on Advancing Nutrition and the Status of Women through Uganda Aquaculture

We will work with Makerere University colleagues affiliated with the Nutrition Innovation Lab to plan and program this conference -- Professor Bernard Bashaasha (Nutrition Lab project PI), Dr. Turyashemererwa (Project Coordinator), and others from the Nutrition Lab. In particular, we will address the potential benefits of broader availability of lungfish as a food item in Uganda. The meeting will involve at least 30 participants from women's farming groups, cooperatives, women-led fish-related business, fisheries students, and others in the value chains for tilapia, clarius, and lungfish. Gertrude Abalo, the Principal of the Fisheries Training Institute (FTI)[2] in Entebbe, Uganda will be enlisted to guide and host this conference. As an institution focused on aquaculture and fisheries with a high proportion of women in the student body, this school will benefit from leading the conference and liaising with AquaFish institutions.

The event will target the leadership of cooperatives and women's organizations. We also foresee an anticipatory session or a pre-meeting event to the Annual Fish Farmer Symposium and Trade Show in February 2017. Presentations will provide an overview and perspective on gender issues in aquaculture and institutional participation that advance women and address gender equity in value chain engagement. We envision one focus on the development of the value chain of emerging species such as the lungfish. Training on value chains and marketing in aquaculture, although addressed to a broader audience, this training will feature women's involvement in the value chain and women as primary producers of fish in Uganda. We envision this a cross-cutting activity on the project as marketing studies, consumer preference, cell-phone applications, and the results of new species development all bear on the topic. We envision a meeting that outlines the way forward in industry development and market expansion for Uganda fish farmers. One study summarizing women's involvement in value chains reported that successful women entrepreneurs felt additional skills were still needed to build their competency, especially more integrated business, and leadership skills.

Activity 2: Conduct an edition of the Annual Fish Farming Conference and Trade Show with a focus on women in aquaculture

The Annual Fish Farming Conference and Trade Show is an activity that this project will reinforce and infuse with gender-related activities to engage all participants in the ways that women can advance aquaculture in Uganda. 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.

---

[2] Fisheries Training Institute (FTI _provides comprehensive instruction to increase fish production and improve utilization of fish and fish products. It is a Regional Institute, one of few such Institutions in Africa. The Institute sits on 9.3 hectares of land on Bugonga point, near the Entebbe airport about 2½km from the main road at Lake Victoria Hotel.

Three presentations to fish farmer cooperatives will address technical needs and issues, as well as specific gender-related concerns in the operation of farmer associations. The fish farmers outside Kampala are often overlooked by project activities.

Trainings and Deliverables

Item	Mechanism (e.g. podcast, reports, factsheets).
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
Presentations to fish farmer cooperative members and leaders	Fact sheet for leaders and participants in cooperatives with emphasis on lungfish as a new market item

Schedule

Activity	2016		2017			
	3rd	4th	1st	2nd	3rd	4th
Conference on Women in Uganda Aquaculture		x	x			
Training on value chains and marketing in aquaculture		x				
Fish farming conference and trade show			x			
Presentations to fish farmer cooperatives			x	x	x	
Report writing, workshops, journal article		x	x	x	x	x

TOPIC AREA

FOOD SAFETY, POST HARVEST, AND VALUE-ADDED PRODUCT MANAGEMENT

❖

ENHANCING FOOD SECURITY AND HOUSEHOLD NUTRITION OF WOMEN AND CHILDREN THROUGH AQUACULTURE AND CAPTURE FISHERIES IN CAMBODIA AND VIETNAM IN THE DRY SEASON

ASIA PROJECT: CAMBODIA & VIETNAM
US Project PI: Robert Pomeroy, University of Connecticut – Avery Point
HC Project PI: So Nam, Inland Fisheries Research and Development Institute

Food Safety, Post Harvest, and Value-Added Product Management/Study/16FSV01UC

Collaborating Institutions and Lead Investigators

University of Connecticut-Avery Point (USA)	Robert Pomeroy
Inland Fisheries Research & Development Institute (Cambodia)	So Nam
	Chheng Phen
	Touch Bunthang
Can Tho University (Vietnam)	Tran Thi Thanh Hien

Objective

The objective of this investigation is to understand the food safety and nutrition security of women and children by analyzing the nutrient density of commonly consumed fish and other aquatic animals (OAAs) from capture fisheries and aquaculture and its products in the dry season and proposed wise nutritional recommendations for adaption options and strategies for improving household food security and nutrition of vulnerable women and children in Cambodia and Vietnam.

Significance

Population growth and other changing variables, such water flow (hydrology), sedimentation (nutrient transport), salinity, and other human activities (e.g., hydropower development dams) are said to affect fish production, livelihood opportunities, food security, nutrition and its implications for human health (MRC, 2009; Sriskanthan & Funge-Smith, 2011; and Halls, 2012).

The prevalence of malnutrition among women and preschool children (under age of five years) continues to be a major problem in Cambodia. The Cambodia Demographic Health Survey 2014 (CDHS, 2014) showed that a high level of protein/energy malnutrition among children under five years of age, with a stunting prevalence rate of 32% (severe stunting -3SD was 9%). The prevalence of wasting was 10 % (severe wasting -3SD was 2%) and the prevalence of underweight was 24% (severe underweight -3SD was 5%), (CDHS, 2014). The CDHS 2014 indicates that more than 4 in 10 mothers are anemic (about 43.9%) and an overall 53 % rate of anemia among children under age 5 years. The prevalence of vitamin A deficiency among women and children are a major public health problem (CDHS, 2014).

Based on the research of the third phase of the AquaFish Innovation Lab (2013-2015), fish and aquatic animals (frog, crabs, snails, and shrimp) are the second largest staple food for women and children in Cambodia with consumption of 145.3 g/women/year and 53 g/child/year in the rainy season, respectively, after rice (Bunthang et al., 2015). They form an integral part of the diet of millions of rural Cambodians. Poor households in particular, with little alternative food production capacity, turn to such sources not only for additional food for themselves as source of their food security, but also for their daily nutritional requirement and for sale to earn income. It is reported that these fish and aquatic animals, particularly small indigenous fish species, have high nutrient content and high bioavailability of micronutrients (Roos et al., 2003, 2006, 2007). Fish, OAAs and products are the major contributor of energy and nutrients from an animal source, providing more than three-fourths (80%) to the total animal protein intake of women in the rainy season (Bunthang et al., 2015). Fish, OAAs and products are also the major contributor to energy, fats, carbohydrate, iron, zinc, calcium, and vitamin A of women, and contributed 69.7%, 54%, 99%, 74.5%, 44.6%, 83% and 87.4%, respectively. Fish, OAAs and products are the largest contributor to the total daily energy and nutrient intake from animal food source, providing around 80% to the total animal protein intake, of children in Cambodia. Fish, OAAs and products were also the major contributor to energy, fats, carbohydrate, iron, zinc, calcium, and vitamin A, contributed 72.1%, 60%, 93%, 57.2%, 44.1%, 92.8% and 56.4%, respectively, in the rainy season (Bunthang et al., 2015).

The application of traditional food processing/preservation technologies in Cambodia dates back in the ancient times and these techniques are often used, especially fish (fermented fish, salted, smoked, fish sauces, and fish paste called "Prohoc". These uphold the Cambodian cultural identity. Fish processing provides many with a continuous source of protein throughout the year. Moreover, the fermentation process of some foods have potential to improve its nutritional qualities, reduce anti-nutrients, decrease pH, increase minerals and provide potential pro-biotic effects through lactic acid bacteria.

The availability of fish and aquatic products in Cambodia should normally be adequate for a balanced diet, but productive capacity or purchasing power of many households is limited, and in these circumstances the diet become more restricted to fish. Trade and market impacts in production levels is said to be changed throughout the value chain such as reduced trade volume and values reduced export earnings and reduced livelihood opportunity and increasing malnutrition.

Seasonality, ecological zones, family size and food distribution among the family members, especially seasonal differences, are the influential indicators affected to food consumption pattern of Cambodian individuals and households especially women and children.

During the third phase of AquaFish Innovation Lab (2013-2015) the study of food and nutritional consumption on women and children in Cambodia was conducted in the rainy season under the UConn's 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 change in 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. Morever, information of consumed fish and other aquatic animals (OAAs) from capture fisheries and aquaculture on women and children in the Mekong Delta of Vietnam is limited. This investigation will focus on this issue. A better understanding of the food and nutritional consumption of women and children can lead to support for both the public and private sectors to increase trade and investment in aquaculture in the Lower Mekong basin.

<u>Quantified Anticipated Benefits</u>

- Two Master (all females) and 3 Bachelor (all females) students will be involved in this investigation. The investigation will support for their university fee and theses.
- At least 6 IFReDI and Can Tho University staff will be involved in the survey such as the project preparation, data collection and training activities.
- At least 1000 IFReDI/FiA and Can Tho University staff, scientists, researchers, government officers and managers, and NGOs will understand the nutrition contexts especially in women and

children in Cambodia through in series of consultation meeting/workshop; and sharing research result findings such as policy brief and technical report.

- At least 450 women and 450 children will benefit from the project by improving nutritional status through the proposed nutritional recommendations for adaption options and strategies in Cambodia and Vietnam.
- Many other people in the Lower Mekong Basin region will benefit from this project through sharing of research findings.

Research Design and Activity Plan

Location

Inland Fisheries and Development Institute (IFReDI), Phnom Penh, Cambodia. The field activities will be undertaken in Upstream Mekong (Stung Province), Downstream Mekong (PoeumRour, Prey Veng province), and Tonle Sap area: (Thom province) in Cambodia; and An Giang province in Vietnam.

Methods

The project activities are organized using a systematic, stepwise approach from collection of information on utilized and analyzed foods, followed by promotion and dissemination of the results, and development of recommendation policy. The activities are conducted by a multi-disciplinary research team, using appropriate quantitative and qualitative research methods.

Activity 1. To assess food and nutrient intakes by women and children; and identify commonly consumed fish and other aquatic resources, aquaculture and its products in the dry season in Cambodia and Vietnam.

Stung Treng province (Upstream Mekong River); Prey Veng province (Downstream Mekong River); and Kampong Thom province (Tonle Sap Area) in Cambodia and An Giang province in Vietnam will be selected for study sites. The data collection will conduct in the dry season (November 2016 to May 2017). The target of the study subjects are women and preschool children (aged six months to five years old). Three hundred (300) eligible women and 300 eligible preschool-age children will be selected in Cambodia and 150 eligible women and 150 eligible preschool-age children will be selected in Vietnam by using simple randomized sampling from the four provinces. Dietary intake will be examined through face-to-face interview by using a single 24-hour food recall to estimate the amount of food that has been eaten in the past 24 hours (Bunthang et al., 2011). IFReDI will provide the dietary assessment survey training to Can Tho University staff and support the survey activity. Food models will be used to identify food items that have been eaten by the subjects. All food and beverage consumed will be recorded using standard household measurement and electronic scale (precision to 0.1 g). The names of local dishes consumed will also be recorded. The amount of each food item consumed will be estimated from the real food models. Mothers will be asked to show the amount of food consumed by her child, which have then been weighed. All food item consumption of women and preschoolers will be converted to weight in grams and the nutrient content of the foods consumed computed by using the ASEAN Food Composition Table (ASEAN FCT, 2000). Included nutrients for evaluation will be energy, macronutrients (Protein, Carbohydrate and fats), and key micronutrients (i.e., iron, zinc, calcium, phosphorus and Vitamin A). The nutrient intakes of women and preschool children will then be compared to the Recommended Dietary Allowances harmonization in Southeast Asia (Barba, 2008; RENI, 2002.) to determine the level of nutritional adequacy of the food intake to estimate the amount of food that has been eaten. Letters to the village authorities informing them on the conduct of the survey will be sent at least one week before the actual survey. The data will collect by trained field enumerators. The training aims to educate field enumerators how to conduct dietary assessment by using the 24-hour food recall questionnaire; educate the interviewers to get familiar with fish species; reinforce the recall interviewers with exercise practices and pilot testing; and educate the interviewers to be familiar with questionnaire before data entry will be employed. Pilot pre-test questionnaire will be conducted in order to identify the potential problems encountered in questionnaires, questions and recall. A letter of the survey objective will be used to inform the local authorities on the conduct of the survey before the actual field work will be started. Questionnaires will be cross-checked by the members of the team for any missing pieces of information followed by data entry. Microsoft Excel 2013 and SPSS Statistics Version 20.0 will be used for data entry and analysis. Data coding, cleaning, and cross-checking will be conducted. Descriptive statistic will be used.

Activity 2. To determine the nutritional composition of nutrient dense fish, OAAs and their products that are identified as commonly consumed by women and children with a focus on macronutrients (protein and fats) and key micronutrients key micronutrients (iron, zinc, calcium, phosphorus and Vitamin A).

The selection of the commonly consumed fish species, OAAs and its products from the result of activity one will be analyzed the nutrient dense. At least 15 of commonly consumed fish species, OAAs, and its products will be analyzed. The selected samples of fish and OAAs will be randomly collected as fresh from different landing sites, local markets, fishermen, and farmers for nutrient-dense analyses, while processed samples will be randomly collected from different market and processors. Proximate composition will use standard methods (AOAC, 1990). Proximate analysis is a quantitative method for determining the macronutrients in food. This includes moisture, total ash content, crude fat, and protein. Moisture will be determined by using a drying oven, ash in a muffle furnace, protein by the Kjeldahl method and fat by Soxhlet extraction. Key micronutrients will be determined by atomic absorption spectrometry or the standard analytical methods of Association of Official Analytical Chemist (AOAC, 1990). Phosphorus, iron, and calcium were also analyzed by AOAC (1990) methods, specifically the molybdovannate method for phosphorus, the hydroxylammonium method for iron, and the muricide method for calcium. The content of non-heme iron will be determined by the widely use ferrozine colorimetric method. Heme iron and complex-bound non-heme iron will be calculated as the difference between total iron and inorganic iron. Microsoft Excel 2013 and SPSS Statistics Version 20.0 will be used for data entry and analysis. The two-way analysis of variance (ANOVA) will be used for the comparison of nutrient density of different selected samples with the statistical significance will be tested using 5% level. The analysis will be conducted at the Industrial Laboratory Center (ILCC), Ministry of industry in Cambodia.

Activity 3. Provide recommendations for policy and strategy development for enhancing food security and nutrition of women and children in Cambodia and Vietnam.

Merely identifying the proposed wise nutritional recommendations for adaption options and strategies will not be sufficient to improve household food security and nutrition of vulnerable women and children in Cambodia and Vietnam. There is a need to provide this information to policy makers, government organizations, NGOs, vulnerable households, especially women and children, to be able to make informed and deliberate decisions on adaptation. As an investigation, the purpose is to generate new information, and disseminate and communicate information generated by the project. Specifically, science-based policy recommendations. This investigation will provide this information through a suite of different communication methods and approaches for each audience.

*Audience analysis***.** The identification of target audiences such as policy makers, government organizations, and NGOs in the fields of fisheries and aquaculture and food and nutrition security as well as poor, rural households who produce fish, and vulnerable women and children.

Project products. The project documents will be reviewed to extract key messages to be presented in the communication products. There will be a scientific-based policy recommendation, two protect technical reports and factsheet in English and Khmer.

Communication and dissemination strategy. The communication strategy is a combination of approaches, techniques and messages to reach different audiences. This will be done through workshops and meetings as well as training of field staff, and home-based providing materials as nutritional education materials for women and mother.

Trainings and Deliverables

Trainings

- Short-term trainings - IFReDI and Can Tho University staff will be trained on dietary assessment by using the 24-hour food recall, fish species, food safety, nutrition, and data cleaning and entry. The training will be conducted at IFReDI, Cambodia and Can Tho University, Vietnam. The training will be conducted between October-November, 2016;
- Long-term trainings-Two masters students and 3 Bachelor students.

Deliverables

The deliverables of this investigation will include (1) final technical report, (2) policy brief recommendation and (3) factsheet in English and Khmer.

Schedule

Activities	Sub-activities	Year	2016									2017												2018	
		Month	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
1. Project Design, Management and design	Review project document		x																						
	Project financial arrangement		x	x																					
2. To assess food and nutrient intake and identify commonly consumed-fish and other aquatic resources by women and children in Cambodia.	1. Design survey			x	x																				
	2. development, training, pilot testing, refinement of survey questionnaire				x	x																			
	3. undertaken the survey						x	x	x	x															
	4. database development and training									x	x														
	5. data encoding										x	x													
	6. nutrient calculation and data analysis											x	x	x	x										
	7 interpreting the result and report writing														x	x	x								
3. To determine the nutritional composition of nutrient dense identified commonly consumed-fish and other aquatic resources, aquaculture and its products.	1. Sample selection and preparation																x								
	2. Conduct analysis																x	x	x	x					
	3. Interpreting the result and report writing																			x	x				
4. Analysis of recommend policy strategy for women and children and the communication product outreach for improving women and children	1. Consultation meeting with women and local agency in Stung Treng, Kampong Thom and Prey Veng																				x	x			
	2. Proposed the recommendation for adaption options and strategies for women as policy recommendation																					x	x		
	3. Communication product outreach																						x	x	
	4. Writing and submission of final technical report																							x	x

Implementing and Assessing Cell-Based Technical and Marketing Support Systems for Small and Medium-scale Fish Farmers in Uganda

Africa Project: Kenya & Uganda
US Project PI: Joseph J. Molnar, Auburn University
HC Project PI: John Walakira, National Fisheries Resources Research Institute

Food Safety, Post Harvest, and Value-Added Product Management/Study/16FSV02AU

Collaborating Institutions and Lead Investigators

Auburn University (USA)	Joseph J. Molnar Claude Boyd
National Fisheries Resources Research Institute (Uganda)	Gertrude Atukunda Moureen Matuha John Walakira
Makerere University (Uganda)	Theodora Hyuha

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. Introduce mobile-based application to the network of agencies and organizations that support aquaculture.

Significance

Previous AquaFish research (13BMA04AU) identified some of the challenges and limitations to the use of mobile phones by fish farmers in Uganda (Matuha 2015). The next step in this work is to engage a mobile application developer in Uganda, then develop, test, and evaluate a prototype app in three target districts.

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 social-networking rapid expansion in developing countries shows the growing role of cell phones in life, business, and culture (Kwaku & Kweku, 2006). Our technical provider in Uganda, AgroMarketDay[3] has an established platform for providing marketing and technical services. Our project extends these possibilities to fish farmers in Uganda.

Several different business models have emerged in efforts to provide technical support to African farmers with cell phones. Each varies in the level of public sector control, business model, cost, and flexibility. One **commercial model** invites farmers to subscribe to a fish-focused network of producers managed by a service provider who moderates the transactions and may be compensated by subscription fees,

[3] http://www.agromarketday.com/

transaction fees, or commissions. The entrepreneur firm builds and supports a network of suppliers, producers, and buyers whose transaction costs support the network. The source of technical information is NaFIRRI and the published literature; the responsiveness to technical questions may be rapid because the entrepreneur is motivated to keep and grow the number of participants. This is the approach we take in Uganda.

A **nonprofit model** may offer subscription services to producers through local lead farmer equipped with advanced, capable cell phones. The organization maintains a central source of technical guidance supported by external donors. The quality of technical information may be good, the advice timely, but the business model for the service may not be sustainable.

A **government-based model** may provide sustainable service to producers through subscription or text-fees. Responsiveness may be limited by budget constraints. Given appropriate location of the network managers, technical competence could be high, the farmers might receive clear signals about government policies, and input and output price discovery processes may be transparent and efficient.
Each of the aforementioned models of market and technical support to fish farmers is emerging in Uganda. The purpose of this activity is to implement and assess the relative efficacy and bread of participation of an application and subscriber network in the rapidly changing Uganda context. A commercial provider has been engaged, the initial steps have been taken, and the new work will implement, assess, and install a useful and sustainable market and information tool in Uganda.

Quantified Anticipated Benefits

- A farmer friendly cell-based aquaculture information system will be readily available for use by fish farmers.
- Farmers with capacity to use mobile phones able to access aquaculture and market information.
- Improved access to print and electronic aquaculture information by farmers.
- Efficient utilization of cellphones by fish farmers to improve fish farming enterprise and their livelihoods.
- Open avenues (workshops, conferences, seminars, Barazas[4], and farm tours) for aquaculture information sharing among farmers, researchers, policy makers and ICT professionals.

Research Design and Activity Plan

Location

Likamis Software Limited in Kampala, a Uganda technical provider, has offices in Kampala. The application will be initially fielded in three districts of Uganda (Wakiso, Mukono, and Mpigi).

Methods

Research task 1. Implement a cell-based system to enable fish farmers to access fish production and market information.

Implementing Partner. We will rely on a Uganda-based contractor with established connections to the major cell phone provider to implement the application. We employ one or more leading farmers in a district to support other farmers. Likamis Software Limited has a mobile platform called AgroMarketDay

[4] Term refers to a community meeting usually convened on the District level where political and technical leaders are held accountable through open discussion with gathered constituents, the *wanainchi*, i.e., ordinary people, the public.

already used by farmers and agricultural stakeholders.[5] The mobile application enables small holder, medium scale and large scale farmers to sell their agricultural produce directly to buyers in any part of the country, without heavily relaying on the rotational community markets. Such venues, where farmers bring their produce to sell, are only open on specific days. Middle men often charge high commissions or offer low prices for the farmers' produce. Selling through a mobile application helps farmers avoid the cost of transporting their produce to and from these markets.

The aquaculture sector in Uganda is composed of a combination of both scattered small-scale producers, a handful of commercial-scale pond-based producers, as well as an emerging set of large-scale producers from cages in Lake Victoria and other Uganda water bodies. This poses a marketing challenge to the producer with very scanty knowledge about available selling opportunities, which often hinder new entrants because of unknown markets and prices. The application will alleviate this constraint by profiling and connecting buyers (fish traders), processors, value addition traders and input suppliers in the central region. We identified a target district where the application is going to be tested and participants enrolled in the system associated with the mobile application.

Mobile applications can help farmers to access reliable farm inputs—feed, fingerlings, nets, and other basic elements of fish culture. Vetted information platforms can reduce the prevalence of misrepresented agricultural inputs in the market. Through the same application, farmers are able to get daily market prices of agricultural commodities across the entire country. Producers are able to price their produce appropriately, and they are also able to learn of better practices conducted in other agricultural sectors.

The Mobile Application. The AgroMarketDay mobile application was copyrighted on 20th October 2014. Once all of the modules for the aquaculture project have been developed, this module will be integrated with the existing suite of applications called AgroMarketDay. Vendors and buyers must be enrolled; farmers must subscribe.

With vendor and producer data in the system, an algorithm within the application matches the farmer to the right buyers in the network. To help farmers determine the right price for their fish, the application enables farmers to view the prevailing fish market prices on offer in the region and beyond. Another challenge that the application addresses is scanty knowledge about available quality inputs. The application links farmers to quality input sources through their mobile devices.

One of the major bottlenecks to aquaculture is inadequate technical guidance to farmers and exploitation by self-proclaimed experts or consultants who purport to advise farmers. The application will address this challenge by implementing eight (8) technical modules for fish farming; site election for both ponds and cages, pond construction, water management, stocking, feeding, harvesting, disease management, and predator control.

For example, subscribing farmers will be able to get expert advice about their diseased fish by using the application; to fill out a form, attach a picture and upload it to our system where an expert will advise the

---

[5] We invited three known application developers to present proposals for developing and implementing a mobile-based system for fish farmers. We elected the AgroMarketDay model based on its coherence, established business experience, and potential for expansion and coverage.

farmers accordingly. This also will ameliorate the problem of inadequate extension support because farmers can access services through their phones.

The application will run on two platforms: USSD for basic phones and Android for smart phone users. The system relies on an algorithm that automatically matches the farmer to the appropriate buyer. When the farmer uploads a notice of product availability using either the smart phone app or the USSD version for basic phones, the algorithm will look through a database to find buyers that match what the farmer is selling. Notice is automatically sent back to the farmer and to all the matching buyers. Messages pass through Cloud messaging to smart phone users and through SMS to basic phone users. Because of the diversity of languages in the country and the literacy levels of the farmers, the application will be translated into five local languages. Most farmers with education are literate in basic English.

Testing and Launching. We will identify a network of fish farmers in the central region from already existing networks such as fish farmer's associations, organization, NaFIRRI and government entities dealing in aquaculture. Trainings will teach farmers on how to use the application to access its services and the benefits that the application will bring to them. A support farmer engaged by the application developer will recruit and assist users in each of the target districts.

Business Model. This project will support the development and implementation of the mobile application. Fees paid by farmers to subscribe to the network, use it for seeking information, and conduct market transactions create revenue for AgroMarketDay. Fish buyers and input vendors pay transaction fees for fish sales and access to producer networks. The fish buyers and input vendors also pay a subscription fee in order to become a verified member in the system. AgroMarketDay engages the support farmers who spread, promote, and facilitate use of the application. The firm has such arrangement for other crops and commodities in Uganda.

Technical Considerations. Uganda has one of the strongest cell networks on the African continent. MTN maintains that it offers 4G coverage across the country. Battery charging is widely available through conventional power systems as well as street vendors offering immediate services.

Google's Mobile-Friendly Test will be used to analyze the application on several criteria and measure its mobile-friendliness. Although a machine assessment, it provides understanding of capability and usability based on a standard test. Another useful tool, offered by Google inside of its Chrome browser, is a mobile emulator. The F12 key (or Ctrl+Shift+I) opens up the developer tools in Chrome. This tool will facilitate migration of web-based information in mobile applications.

Research task 2. Conduct trials of cell-based aquaculture applications for fish farmersIntensive individual and group interviews will be conducted with fish farmers, fish farmer's association leaders, researchers, and representatives from public agencies. We use snowball and positional sampling techniques to identify individuals serving the aquaculture sector in Uganda--MAAIF, NARO, and DFOs. In addition, local leaders and IT professionals will be asked about how cell-based information systems can address aquaculture information topics. Such topics include; stocking, harvesting, feeding management, pond construction and management, disease management, water quality management, broodstock management and market prices. The interviews will be conducted in the selected districts of Uganda.

The meetings will present the application as it has been developed, as well as other state-of-the-project information. The intent is to gauge and record participant perspectives on the productive use and development of mobile applications for fish farmers. The guided conversations will endeavor to identify critical aquaculture information needs and mobile phone access preferences of fish farmers.

We will employ specialized software (NVivo, Atlas-ti) to process and summarize information obtained through these conversations. The tool will be piloted among selected fish farmers (40), suppliers of inputs (10), and extension service providers (5). This piloting will involve training the users to employ the application on their mobile phones to access marketing information, technical support, price discovery, and input sourcing. Informal discussions will be held with targeted populations of fish farmers who participate in the trainings on the use cell-based aquaculture information application

Two of the discussion groups will be largely women and the focus of these meetings will address the differential needs and experiences of women. Access to cell phones, the experience of using cell-based information, and expectations for the technology will be addressed. We will subsequently utilize information obtained from fish farmer discussions about the cell-based technical and marketing support system to shape application development. Simple queries about use, experience, and expectation will be used to profile patterns of uptake and participation in mobile-based technical and information systems.

Research task 3. Introduce mobile-based application to the network of agencies and organizations that support aquaculture. We will hold three workshops in Jinja, Kampala and Gulu that will involve project participants, selected officials from NARO, MAAIF, researchers and ICT technical personnel. These will mainly focus on the introduced mobile application. The meetings will gauge partner responses, and assess the modules that have been developed for cell-based system. In particular, the interaction between business model, technical responsiveness, and user-friendliness will be addressed.

Mechanisms for continued involvement of the public agency fish farmer support network will be discussed. In particular, we seek to open avenues (workshops, conferences, seminars, Barazas, and farm tours) for aquaculture information sharing among farmers, researchers, policy makers and ICT professionals. The workshop report will summarize participant comments and issues identified in these meetings. These meetings are a central aspect of our exit strategy in Uganda.

Trainings and Deliverables.

Item	**Mechanism** *(e.g. podcast, reports, factsheets)*
Profiled information packages on fish buyers, processors, value addition, fish farmers and input suppliers	Two Workshops will be held in Kampala
Training of farmers to use the newly developed aquaculture app	Two trainings of farmer will be held in Kampala
Specific cellphone aquaculture information packages.	Inventory sheets, Brochures, farmer leaflets
Cell-based information application system for fish farmers	Aquaculture App
Report on implementation and evaluation of usage of a cell-based aquaculture Application by fish farmers.	Journal article

Schedule

Task	2016		2017			
	3rd	4th	1st	2nd	3rd	4th
Hold workshops with fish buyers, processors, value addition	x	x	x	x	x	x
Field implementation of cell-based application Hold workshops with fish farmers and input supplier		x	x	x	x	x
Conduct two interviews and two discussion groups with fish farmers in Wakiso, Mpigi, and Mukono			x	x		
Develop cell-based aquaculture application		x	x	x	x	x
Train farmers to use aquaculture application				x	x	x
Hold workshops with public agency fish farmer support network. Evaluate utilization of cell-based application					x	x

TOPIC AREA

POLICY DEVELOPMENT

❖

GUIDANCE AND POLICY RECOMMENDATIONS FOR SUSTAINABLE SNAKEHEAD AQUACULTURE AND AQUATIC RESOURCE MANAGEMENT IN CAMBODIA AND VIETNAM

ASIA PROJECT: CAMBODIA & VIETNAM
US Project PI: Robert Pomeroy, University of Connecticut – Avery Point
HC Project PI: So Nam, Inland Fisheries Research and Development Institute

Policy Development/Activity/16PDV01UC

Collaborating Institutions and Lead Investigators

University of Connecticut-Avery Point (USA)	Robert Pomeroy
Inland Fisheries Research and Development Institute (Cambodia)	Hap Navy
Can Tho University (Vietnam)	Truong Hoang Minh

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.

Significance

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.

This new proposed project, along with the previous AquaFish CRSP and Innovation Lab projects, has been conducting research to support sustainable snakehead aquaculture in the Lower Mekong Basin of Cambodia and Vietnam and the lifting of the snakehead ban in Cambodia. The AquaFish CRSP project produced a number of outcomes, including development of a plant based feed for snakehead fish; recommendations to the government of Cambodia and the private sector for a sustainable snakehead aquaculture industry; value-added products for women from small sized/low value fish such as fish paste and fish sauce; extension/outreach technologies; recommendations for improvements in the trade and marketing system for both capture and culture fish in the region; and recommended policies to improve management of small-sized/low value fish in the Mekong area. Through the AquaFish Innovation Lab research, the laboratory trial and on-farm trial on the use of immunostimulant in snakehead diets based on soy protein concentrate and fish meal, evaluating survival, growth and immunology of fish immunology was completed. The snakehead farmers were all women who were selected with the aim to improve their snakehead rearing technique. The project developed methods for snakehead products conservation and processing (using salt supplemented with and without Bromelain and dried fish processing with and without sugar were done successfully). Once the protocols for snakehead value-added products (fermented and salted dried snakehead products) were in place, the methods were transferred to women snakehead farmers in An Giang province through two short training courses with the participation of 60 women farmers. Wild and hatchery-stocked brooders of snakehead were stocked and conditioned in hapas at FARDeC in Cambodia for induced spawning. Induced spawning, weaning experiment and grow-out experiment has been completed. First generation (F1 adapted to formulated feed) of wild snakehead have been stocked and conditioned in hapas at FARDeC. The project has identified commonly consumed-fish and other aquatic resources, aquaculture and its products in Cambodia, and determined the nutritional composition of nutrient dense identified commonly consumed-fish and other aquatic resources by women and children with key micronutrient such as iron, phosphorus and calcium; and macronutrient (i.e., protein and fat). These outcomes have impacted or are impacting both the private and public sectors through improvements in technologies, commercialization of new aquatic products, sustainable aquatic resource management practices, and policies for aquaculture and capture fisheries.

This activity utilizes this broad research on snakehead aquaculture to support the development of sustainable snakehead aquaculture in Cambodia through research based 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.

Quantified Anticipated Benefits

- 50 scientists, researchers, resource managers, government officials, and non-government organizations 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.
- 500 snakehead farming households in Cambodia will be better informed on the development of sustainable snakehead aquaculture through research based guidance on feeding, breeding, weaning and rearing/grow-out.
- 100 women involved 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.
- Five researchers in Cambodia and Vietnam will be trained and have experience on using economics to analyze specific impacts of cost and profitability of snakehead culture.
- This investigation will provide return benefits to the US by allowing the Lead PI to expand his work in SE Asia on food security and fisheries and climate change and return this information and knowledge to graduate students in the University of Connecticut.

Research Design and Activity Plan

Location

The activity will be undertaken in four provinces of Cambodia (Kandal, Kampong Chnang, Kampong Thom, and Siem Reap) and four provinces in Vietnam (An Giang, Dong Thap, Vinh Long and Tra Vinh). The geographic area of focus for the four provinces in Cambodia is in and surrounding the Tonle Sap Lake and Mekong-Bassac areas. The four provinces in Vietnam include three on the Mekong River (two of which border Cambodia) and one on the Mekong River on the coast.

Methods

To support sustainable snakehead aquaculture there is a need to provide science-based information to government and aquaculture 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 this information through a suite of different communication methods and approaches for each audience.

Activity 1. This activity will examine the cost and profitability for snakehead culture in different production systems in Cambodia and Vietnam. The activity will build 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 activity will focus on snakehead culture in cage and pond production systems at different scales of production (small, medium, large). An extensive review of past studies will be carried out in relation to production cost and profitability of snakehead culture in Cambodia and Vietnam. The review and desk-based research will 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.

Activity 2. Audience analysis. The identification of target audiences (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.

Activity 3. Project products. The project documents from all of the activities of the AquaFish CRSP and Innovation Lab projects will be 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 will be 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:

1) Snakehead feed
2) Snakehead feeding strategies
3) Processing and value-added products for women
4) Improvements in the trade and value chain for both capture and culture fish in the region
5) Human nutrition and human health impacts of fish
6) Wild and hatchery-stocked brooders of snakehead stocking and conditioning
7) Snakehead breeding, weaning, and rearing/grow-out
8) Economics of production

Trainings and Deliverables

- ***Final national workshops***: one workshop will be organized in Cambodia to present the final findings of the CRSP and Innovation Lab projects;
- ***Policy briefs***: two science based policy briefs in local language;
- ***Guidelines/best practices***: two guidelines/best practices documents on sustainable snakehead aquaculture;
- ***Posters/leaflets/factsheets***: Information about the project and findings of the project will be prepared, printed and delivered popularly in two posters, two leaflets, and two factsheets in local language;
- ***Websites***: The project will utilize IFReDI, Can Tho University and University of Connecticut websites to exchange information;
- ***Papers***: at least one paper will be published in either a national or international journal
- ***Long-term training***: At least 3 undergraduate students: Cambodia and Vietnam and 1 MSc student on the economics of production of snakehead aquaculture.

Schedule

The starting date is 1 March 2016. The completion date is 28 February 2018. Detailed activities plan as in table below:

Activities	**2016**				**2017**				**2018**
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
Collecting and reviewing secondary information on economics of snakehead production systems		X	X						
Analysis and write up economics report				X	X				
Audience analysis				X	X				
Review of project products and identification of key messages						X	X	X	X
Communication and dissemination strategy						X	X		
Development of products				X	X	X	X	X	
Dissemination of products								X	X
Final workshops									X

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

AFRICA PROJECT: GHANA & TANZANIA
US Project PI: Kwamena Quagrainie, Purdue University
HC Project PI: Stephen Amisah, Kwame Nkrumah University of Science and Technology

Marketing, Economic Risk Assessment, and Trade/Activity/16MER01PU

Collaborating Institutions and Lead Investigators

Purdue University (USA)	Kwamena Quagrainie
Kwame Nkrumah University of Science & Technology (Ghana)	Stephen Amisah
FarmerLine (Ghana)	Alloysius Attah

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.

Significance

During the 2013-2015 funding cycle, AquaFish Innovation Lab funded an investigation that developed a cell-phone based Fish Market Information System (FMIS) with a focus on tilapia and catfish in Ghana. This is because tilapia and catfish are the dominant fish species that are farmed and caught in inland waters. The FMIS has a database of current farm-gate and market prices of the two species in selected locations in Ghana assembled by fisheries officers and selected agents. 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. 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 either 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.

AquaFish CRSP and AquaFish Innovation Lab activities in Ghana over the years have addressed only aquaculture issues and not the capture fisheries sector. This investigation seeks to address the biased focus on aquaculture as the artisanal fisheries sector contributes significantly to seafood supply in Ghana. AquaFish Innovation has also funded a cell-phone based project in Uganda, that developed baseline information about the needs and interest of fish farmers that could be used by public agencies, NGOs, and cellular providers to develop services for fish farmers. In Kenya, the Kenya Marine and Fisheries Research Institute's (KMFRI) Enhanced Fish Marketing Information System (EFMIS) was piloted with a select group of fish farmers. Fish farmers were trained to query the EFMIS database to enable them become familiar with how the system worked, i.e., how to access Lake Victoria's fish landing information. This investigation will leverage the knowledge gained from the AquaFish funded Uganda and Kenya projects through collaborative work so that the countries can learn from one another in the development of this technology for the aquaculture sector. If well developed and implemented, the cell phone technology can improve the livelihoods of fish farmers and agents along the fish value chain by narrowing market information gaps and improving networking along the chain. With improved communication along the fish value chain, there would be better market efficiencies and reduction in transaction costs. This would result in improved incomes and household purchasing power for fish farmers and fish retailers. With more incomes, these households can afford to buy more nutritious foods to improve household health.

The UN Millennium project included eight development goals that committed nations to global partnerships to reduce all aspects of extreme poverty including income poverty, hunger, disease, lack of adequate shelter, and exclusion, while promoting gender equality, education, and environmental sustainability. Though the Millennium Development Goals (MDGs) do not make specific reference to fisheries and aquaculture development, those sectors are targets for development to alleviate hunger (MDG #1) and address environmental sustainability issues (MDG #7).

There are two capture fishery sectors in Ghana: marine (sea and lagoons) and inland (lakes, rivers and reservoirs). The marine fishing industry has three main sectors: Artisanal or small scale, semi-industrial or inshore, and industrial subsectors. The artisanal fisheries sector is the most important in terms of landings and contributes approximately 80% of the total marine fish production (Mensah & Antwi, 2002; Amador et al., 2006). The artisanal fisheries sector is reported to employ about 20% of the nation's labor force, or about 2 million people (Atta-Mills et al., 2004). It is estimated that the marine fishery sector accounts for about 3.9% of Ghana's gross domestic product (GDP) and 11% of the Agriculture GDP (Bank of Ghana, 2008). The total landings from inland fisheries constitute approximately 10% of the total national landings of capture fisheries, with the remainder coming from the marine fisheries. The inland fisheries are all artisanal operating from about 1,232 fishing villages along the shores of the Volta Lake (Braimah, 2003).

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 includes 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 are dependent solely on inland and marine resources and their contribution to the national food system will require appropriate investments in developing the seafood value chain to reduce the waste, enhance efficiency, and strengthen value addition. Therefore, broadening the applicability of the existing FMIS to include the marine artisanal fisheries subsector will go a long way to improving the welfare of artisanal fishermen through a reduction in transaction costs and improvement in the benefits from fish trade.

The FMIS at this stage is a pilot technology that functions with a focus on tilapia and catfish. The services it provides help to address market information asymmetries between buyers and sellers of tilapia and catfish, and is helping to improve the bargaining power of smallholder fish farmers/fishers in their interactions with fish traders. The system is enhancing the efficiency of input use and increasing the size of the average fish by delivering concrete suggestions and market information through mobile messaging. These benefits are lacking in the marine artisanal fisheries subsector though fish from capture fisheries form part of the whole seafood value chain in Ghana. In addition, it is important to find ways that best allows the benefits of the system to reach many more stakeholders and general fish consumers.

This investigation will expand the functionality of the current FMIS with more value chain services to include prices of inputs, prices of marine species at selected landing sites, and access of the system to consumers. By providing farmers easy access to information on fingerling and feed prices and where to buy them further empowers them to farm efficiently. Allowing consumers of fish access to market prices will further close the gap between what farmers 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 will help fish farmers/fishers, fish processors and traders to more efficiently support urban markets with seafood products. In addition, the improved FMIS will have applicability to the marine artisanal fisheries subsector from fish trade.

Quantifiable Anticipated Benefits

1. A Market Information System that organizes market prices of seven major seafood species in Ghana and serves as a resource for the development of marketing plans and strategies.
2. Access to sufficient seafood market information that is needed for informed market and policy decisions.
3. Database assembled over a period of time will be available to US researchers for any quantitative analysis of the seafood market in Ghana.
4. An information system that can be expanded into a portfolio of agricultural-based and non-agricultural rural enterprises.

Research Design and Activity Plan

Methods

Objective 1: Broaden the applicability of existing FMIS to include the marine artisanal fisheries subsector.

The current market information system in Ghana focuses on only tilapia and catfish prices from different supply points and markets. The marine species that dominate fish catch landed are sardinellas, anchovies, mackerels, red fish, and tuna (Aheto et al., 2012; Amador et al., 2006). ***Farmerline*** will provide additional programming to broaden the applicability which allows marine artisanal fishermen to obtain market prices for 5 major marine and species (Tuna, Dentex [Redfish], Mackerel, Sardinellas, and Caranx) landed in the major markets via voice and SMS/text messaging. Artisanal fishermen will register with the system and can request the market information through a short code. Fisheries officers and agents will be recruited to visit selected landing sites the coastal regions of Ghana as well as major retail markets weekly to obtain prices. Preliminary discussions have taken place with the Ministry of Fisheries and Aquaculture Development (MFAD) on providing assistance through the fisheries officers. FarmerLine also has field officers who will assist with the collection of market prices. A weighted average weekly price will then be calculated and made available to users.

Objective 2: Expand the functionality of the FMIS by customizing market price collection procedure to enable accurate and real time data collection.

Farmerline will provide the services of additional programming to further improve the quality and timeliness of data collected through customization of the MERGDATA platform. The data collection process will be complemented with crowd-sourced information from consumers selected randomly to provide vital feedback on the validity of the prices being received. Thus, there will be new partnerships and expanded scope of engagement with information assembled in the FMIS database. The enhanced FMIS would provide more accurate and timely market information.

Objective 3: Train artisanal fishermen on the use of the Seafood Market Information System.

Two landing sites along the coast, one each in the Greater Accra region and Central region, will be identified as a venue for training. The training activities will be conducted in collaboration with the MFAD, chief fishermen, and elders in the selected fishing communities. The workshops will be publicized through the MFAD and by word-of-mouth in the coastal communities. Regional and District fisheries officers will be involved in the training of the artisanal fishermen.

Trainings and Deliverables

1. Two training programs on how to use mobile phones to receive information on seafood prices and other market data will be offered to artisanal fishermen one each in the Greater Accra region and Central region. It is anticipated that there will be 50 participants at each of the training sessions (total of 100).
2. A database of seven major seafood prices and other market information – Tilapia, African catfish, Tuna, Dentex (Redfish), Mackerel, Sardinellas, and Caranx.
3. An electronic forum for users of FMIS that enhances seafood market activities for small-scale fish farmers, artisanal fishermen, fish retailers and consumers.

Beneficiaries

Fish farmers, artisanal fishermen, fish processors, seafood traders and retailers, consumers, and policy makers.

Future Plans

The plan is to get as many users as possible for the enhanced FMIS. A high patronage of the system makes a pay-per-use more feasible. Widening the scope of information and services available on the FMIS platform will expand usage by many more stakeholders that will include fish farmers, artisanal fishermen, fish processors, seafood traders and retailers, consumers, and policy makers, etc., which then allows for maximum impact through scale and sustainability. The benefits shown to stakeholders using the information will motivate stakeholders to pay, which allows for further development of the system to fit the needs of users. Charges for usage will take into account the cost of system maintenance and programming support. Pricing arrangements will be made with mobile phone companies for long-term financial sustainability of the system.

Schedule

Renew / Amend subcontracts with KNUST and *Farmeline*	August – October 2016
Additional Programming of the electronic information platform	November 2016 – March 2017
Data and information collection from landing sites and market centers in the coastal regions.	April – June 2017
Field testing of the enhanced FMIS	July – August 2017
Training marine artisanal fishermen in the use of FMIS	September – December 2017
Reporting	January – February 2018

ASSESSMENT OF PRICE VOLATILITY IN THE FISH SUPPLY CHAIN IN UGANDA

AFRICA PROJECT: KENYA & UGANDA
US Project PI: Joseph Molnar, Auburn University
HC Project PI: John Walakira, National Fisheries Resources Research Institute

Marketing, Economic Risk Assessment, and Trade/Activity/16MER02AU

Collaborating Institutions and Lead Investigators

Alabama A&M University (USA)	James O. Bukenya
Makerere University (Uganda)	Theodora Hyuha
National Fisheries Resources Research Institute (Uganda)	Moureen Matuha

Objectives

1. Evaluate the factors influencing fish price formation at the farm level (aquaculture) and whether or not fish prices at farm level have become more volatile over time.
2. Examine the price volatility relationships between aquaculture and captured fisheries.
3. Examine the degree of price volatility at the ex-vessel, retail and wholesale market levels, and use the results to forecast future fish prices.

Significance

Price fluctuations in the Uganda fish markets has become one of the main risks faced by fish producers. These price movements are for the most part risky, as the direction and force of the motions are largely unknown on a short-term basis. The economic costs of highly fluctuating prices are not only experienced by fish producers but are transferred to the entire value chain. Wholesalers, retailers and consumers increasingly demand stability of price and supply, and often have little understanding for biological and other mechanisms driving the formation of prices in the market. In view of this, price forecasting is becoming increasingly relevant.

At a basic level, fish prices are volatile because the short-run production and demand elasticities are low. On the demand side, the short-term elasticities are low because the producer price is a small percentage of the final retail fish price while on the production side, short-term elasticities are low because input decisions are made before new output prices are known. It is against this background that we are proposing to study the degree of price volatility in the Uganda fish supply chain.

By defining volatility as the fluctuations of prices above and below some pre-conceived long-term trend or equilibrium, the study will provide an approach to predict next-period fish prices based on the Generalized Autoregressive Conditional Heteroscedasticity (GARCH) methodology.

An increase in price volatility implies greater uncertainty about future prices because the range in which prices might lie in the future becomes wider. As a result, fish producers and consumers can be affected by increased price volatility because it augments the uncertainty and the risk in the markets. More specifically, increased price volatility can reduce the accuracy of fish producers' and consumers' forecasts of future fish prices, thereby causing welfare losses to both fish producers and consumers. It is crucial for these decision makers to be aware of the degree of price volatility so as to be able to adopt appropriate hedging strategies.

The mobile app study (Investigation 2) and the volatility work proposed here are linked in several ways. Firstly, the forecasted prices information that will be generated from the volatility study will be disseminated to fish farmers and other stakeholders through the proposed mobile app. Sharing such information will create awareness among farmers on how well or bad the market is doing at a given period, and will give farm managers a sense of future market prices and economic trends. Secondly, prevailing fish prices on the local markets gathered through the mobile app on a day-to-day frequency will be collected and incorporated into the baseline historical information database upon which future price forecasts will be based.

Quantified Anticipated Benefits

- Stocking decisions in the aquaculture sector are taken on the basis of expected prices at harvest; hence forecasting fish price empowers fish farmers to make informed decisions regarding stocking in the future.
- Increase sales and incomes for fish farmers
- Fish production become more market oriented and reduces post-harvest losses.
- Reduced marketing and other transaction costs for farmers.
- Improved market linkages and farm sales for farmed fish.

Research Design and Activity Plan

Location

Normal, Alabama and Kajjansi, Uganda.

Methods

The proposed approach to precise measurement of price formation and volatility will involve presentation in both non-technical and technical forms.

Research task 1: Non-technical analysis (descriptive analysis***)***

In this analysis a number of different analyses (less advanced econometrically) of the price data will be conducted. The methodology will start by descriptively analyzing the behavior of price volatility in the fishery industry in central Uganda. We will apply several measures of volatility to monthly price data in order to apprehend indications of the properties of volatility that will in turn direct our further analysis. Particularly, we will use the following indicators to understand fish market price dynamics over time:

a. The Mean (arithmetic mean): the sum of all measurements in the period divided by the number of observations in the data set;
b. Volatility (variance): the dispersion of prices from their mean values (mathematical expectation of the average squared deviations from the mean).
c. The Coefficient of Variation: the ratio of the standard deviation over the mean as a measure of the dispersion of individual data. The higher the coefficient, the larger the dispersion and the higher the volatility of prices.
d. The Standard Deviation (σ): this shows how much variation or dispersion exists from the average (mean), or expected value. A low standard deviation indicates that the data tends to be very close to the mean; high standard deviation indicates that the data are spread out over a large range of values.
e. The Moving Average: price data will be analyzed by creating a series of averages of different subsets of the full data set. A moving average is commonly used with time series data to smooth out short-term fluctuations and highlight longer-term trends or cycles. Moving Average techniques forecast prices by calculating an average of actual prices from a specified number of prior periods; each new forecast drops the prices in the oldest period and replaces it with the prices in the most recent period; thus, the data in the calculation "moves" over time.

Examination of the monthly fish price, production and consumption data to determine if certain factors including mobile phone coverage are significant in the formation of fish prices over time. Particularly, we will examine the impact of mobile phone coverage on producer price dispersion across markets. The following relationship will be estimated using OLS regression:

$$P = \beta_0 + \beta_1 \text{Production} + \beta_2 \text{Consumption} + \beta_3 \text{Mobile Phone Coverage}$$

where price (P) is defined as the price of fish while production and consumption are measured in tons (in thousands).

Research task 2: Seasonal price analysis (multiple regression)
Traditionally, farmers need only short-term predicted prices because fish is a perishable product, so this predication can permit some spatial and temporary arbitrage. However, traders and exporters need short, mid and long-term price predictions.

Regression analysis will be used to predict fish prices (the equation's outputs) for short-term (monthly prices), mid-term (quarterly) and long-term (annually) with the incorporation of dummy variables for detailed seasonal analysis (following the impact of particular months and quarters as these are the equation inputs).

A regression function will be specified as:

$$P = \beta_0 + \beta_1 * Month + \beta_2 * Q_{T1} + \beta_3 * Q_{T2} + \beta_4 * Q_{T3} + \beta_5 * Q_{T4}$$

where P is price; β_0 represents the coefficient for the intercept; β_1 to β_5 are slope coefficients; and Q_{T1} to Q_{T4} represent quarters.

Research task 3: Measuring volatility and price forecasting (GARCH approach)
For the aquaculture industry providing information on the volatility of prices is potentially valuable. There is substantial variability in industry profit levels, and an important part of this variability is due to fluctuating prices. Particularly, we will investigate the price volatility in the catfish industry, and thereby obtain information with respect to the nature of price risk that catfish farmers are facing.

To put the aquaculture sector into a broader perspective, we will compare it with capture fisheries. Dahl and Oglend (2014) showed that capture fisheries, which are exposed to both the risks of production (catch volume and type) and market price fluctuations, tend to have more volatile prices than aquaculture, which mitigates some of the production risk, thus lowering volatility.

The working hypothesis is that as aquaculture industry grows and accounts for an increasing portion of Uganda's fish market, overall fish prices should become less volatile. We will measure price volatility by using the generalized autoregressive conditional heteroscedasticity (GARCH) approach by estimating two different multivariate GARCH models, i.e. the DVEC(1,1) and the BEKK(1,1) models.

The analysis will relate asymmetries in the price transmission mechanism (addressed in the last study) and changes in price volatility by incorporating regime variables into the conditional variance covariance matrix of the estimated multivariate GARCH models. The models can be represented as GARCH (p, q):

$$\varepsilon_t = \nu_t \sqrt{h_t}$$

where ν_t is white noise term and

$$h_t = \alpha_0 + \sum_{i=1}^{q} \alpha_i \varepsilon_{t-1}^2 + \sum_{i=1}^{p} \beta_i h_{t-1}$$

defining the conditional variance. Similar work by Buguk et al., (2003) used an exponential GARCH model to test univariate volatility spillovers in the US catfish supply chain.

Trainings and Deliverables

Item	Mechanism ***(e.g. podcast, reports, factsheets).***
Historical price trends in the fish industry	Technical report for extension.
Information on price volatility, price forecast and policy recommendations	Technical report for extension.

Schedule

	2016		2017			
	3rd	4th	1st	2nd	3rd	4th
Data Entry and Cleaning		x				
Data entry and analysis using LIMDEP software		x	x	x	x	
Developing policy recommendations & leaflets					x	x
One presentation to farmer conference			x	x	x	x

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

ASIA PROJECT: BANGLADESH
US Project PI: Russell Borski, North Carolina State University
HC Project PI: Shahroz Mahean Haque, Bangladesh Agricultural University

Mitigating Negative Environmental Impacts/Experiment/16MNE01NC

Collaborating Institutions and Lead Investigators

Bangladesh Agricultural University (Bangladesh)	Shahroz Mahean Haque Mst. Kaniz Fatema Sadika Haque
North Carolina State University (USA)	Russell Borski

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 experiment 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 and produce an extension factsheet outlining the benefits of the new technologies for transfer to farmers, extension agents, NGOs, and other stakeholders.

Significance

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 the value 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 are important steps 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 therefore creates 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, the first experiment of the proposed research will assess 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) suggest 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 reduced feed ration. Here we will assess 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.

Quantified Anticipated Benefits

1. Use of a mixed-trophic level polyculture production system will increase nutrient utilization efficiency and reduce negative environmental and social impacts (pond eutrophication) of producing Koi.
2. We anticipate improvements in environmental water quality for the culture of Koi with carp. Further improvements are likely to occur with reduced feeding, semi-intensive culture practices.
3. Successful implementation of feed-reduction strategies for Koi/carp polyculture will reduce feed costs by as much as 50%, thereby increasing production of high-value crops as farming of these fish will become more attractive to low-income homestead farmers.
4. We anticipate greater production yields (kg) of fish will benefit human nutrition, as it will enhance income and availability of fish for rural farming households. This is particularly true of air-breathing fishes, since Shing catfish are high in micronutrients commonly lacking in the diet of rural Bangladeshis.

5. Overall, we anticipate that semi-intensive culture of high-value Shing catfish with Koi-carp will improve earned incomes through greater yield of fishes and promote more sustainable production of fish with high nutritional value in Bangladesh and other regions of Asia.

Research Design and Activity Plan

Location

These studies will be performed onsite at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Water quality analysis will be performed at the Water Quality and Pond Dynamics Laboratory (BAU). Dr. Sadika Haque at the Dept. of Agricultural Economics (BAU) will conduct the economic analysis for this experiment.

Methods

Experiment 1. Compare combined polyculture of Koi with two major carps (Rohu and *Catla*) versus Koi monoculture under semi-intensive pond culture conditions and assess the effect of reduced feed ration in polyculture of carps and Koi.

Null Hypothesis: No differences in growth efficiency, water quality, or economic returns are observed with koi farming with inclusion of carps under intensive culture practices (no pond fertilization) or semi-intensive culture with fertilization/reduced feeding strategies.

This experiment will evaluate 75% and 50% reduction in daily ration to identify the feeding rate yielding better economic returns for semi-intensive Koi-carp polyculture production. We will test the effects of full or reduced-feeding (2) on growth, production yield, and economic profitability (cost-benefit analysis) to minimize both costs and mortality due to poor water quality. As an additional benefit, this design will also test whether the current practice of Koi monoculture could be better managed (reductions in eutrophication) by the addition of carps. Two Indian carps, Rohu (*L. rohita*) and *Catla* (*C. catla*), will be raised with Koi (climbing perch, *A. testudineus*) in mixed-culture ponds. The feed reduction strategies will be implemented using the following experimental design:

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	0	0	4:1 (N: P)	4:1 (N: P)
Feeding Protocol	Full Daily Ration	Full Daily Ration	75% Daily Ration	50% Daily Ration
Replicates (*n*)	4	4	4	4

This design contrasts the current monoculture intensive practice of Koi farming (T1), against treatments incorporating carps under intensive practices (T2), or culture with reductions in daily feeding (T3, T4). As carp farming requires significant primary production, levels that may not be achieved under restricted feeding, ponds will be fertilized weekly for these groups (T3, T4). As fertilizer is roughly 14% of feed costs, it is anticipated this design will prove more efficient and profitable than with full-feeding alone (T1, T2). The treatment groups will be randomly assigned to ponds (N =16, 100 m^2, 1.5 m depth). Prior to flooding and stocking, the ponds will be dried, re-excavated, and limed (25 g $CaCO_3/m^2$). They will be fertilized initially at 28 kg N and 5.6 kg P/ha prior to stocking. During the production period (120 days), T3 and T4 ponds will be fertilized at a rate of 28 kg N/ha/week and 5.6 kg P/ha/week. Full rations of feed (30% crude protein, commercial grade) will be administered by feeding to satiation, determined empirically every two weeks. Based on the current practice fish are fed 20%-5% bw/day with 5% declines at monthly intervals. The reduced-feeding groups will receive 75% and 50% less feed, based on values derived from T1 and T2. Feed amounts will be recorded for cost-benefit analysis performed at the end of experiment. All ponds will be sub-sampled every 14 days to collected growth data (mean fish length and weight).

Water quality will be monitored daily (dO2, pH, turbidity/ secchi-disk depth), while additional parameters will be measured weekly or fortnightly by the Water Quality and Pond Dynamics Laboratory at BAU: ammonia, nitrates, total phosphate, alkalinity, chlorophyll-a.

Production yields (market weight, kg), estimated market returns, feed input costs (feed, fertilizers, labor, fingerlings), and labor costs will be gathered for all treatment groups at the end of experiment for marginal cost-benefit analysis by Dr. Sadika Haque (BAU).

All treatments will be tested for significant differences in growth (mean length, weight X time), growth efficiency (specific growth rate, feed conversion ratio), and water quality using Analysis of Variance ($p < 0.5$; preplanned contrasts: T1 with T2, T2 with T3 and T4; T3 with T4).

Experiment 2. Assess economic and environmental benefits of combining Shing at different densities with Koi-carp pond polyculture.
Null Hypothesis: No differences in growth efficiency, water quality, or economic returns are observed when Shing are polycultured with Koi and carps, regardless of stocking density.

This experiment will assess whether incorporation of Shing into Koi-carp polyculture could provide additional benefits to farmers as the new polyculture technology could provide an additional crop of high nutritional and economic value. Currently, Shing fetch almost 750 BDT/kg ($5/lb.), a price substantially higher than any other cultured seafood in Bangladesh. While Experiment 1 will directly compare semi-intensive polyculture of Koi with carps versus current Koi monoculture practices, we expect that daily reductions in feed ration by 50% will prove the most cost-effective strategy as was seen with Shing-carp polyculture in our previous AquaFish research. In this experiment we will test different stocking densities of Shing in Koi-carp polyculture under the assumption that the 50% reduced feeding and fertilization strategy will be most beneficial (T4 group in Experiment 1). Should this not be the case then we will test Shing culture under conditions that yielded the best net return on investment from the T2-T4 fertilization-feeding strategy in Experiment 1. The following experimental design is proposed:

Parameter	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/Feeding Best of T2-T4 – Experiment 1, Assumes T4 is best here	4:1 (N: P), 50% Daily Ration	4:1 (N: P), 50% Daily Ration	4:1 (N: P), 50% Daily Ration	4:1 (N: P), 50% Daily Ration
Replicates (*n*)	4	4	4	4

The treatment groups will be randomly assigned to ponds (N = 12, 100 m^2, 1.5 m depth). The rate of feed applied will be calculated based on that established for Koi in Experiment 1, and the amount applied will be calculated based on the biomass of both Koi and Shing and adjusted every two weeks from fortnightly sampling of fish weights. Shing growth is relatively slow and fish reach marketable sizes at 25-60 g, hence the reason for their high price. Application of the additional feed for production of Shing should therefore have minimal impact on water quality or in reaching pond carrying capacity requiring costly aeration and water exchange. The preparation of ponds, fertilization rates, and sample collection (growth data, water quality parameters) will be performed as described in Experiment 1. As outlined in Experiment 1, the final production yields (market weight, kg), estimated market return, feed and labor costs will be determined at the end of experiment for an additional cost-benefit analysis by Dr. Sadika Haque (BAU). Treatments will be tested for significant differences in growth (mean length, weight X time), growth efficiency (specific growth rate, feed conversion ratio), and water quality using Analysis of Variance ($p < 0.5$).

Trainings and Deliverables

1. The findings from Experiments 1-2 will be reported through the Technical Reports of the AquaFish Innovation Lab (Final Investigative Report, FIR), and possibly through scientific proceedings of regional and/or World Aquaculture Society meeting. Depending on results, a peer-reviewed paper will likely follow completion of the project.
2. We estimate two-four undergraduate and graduate students will receive training on management strategies related to Shing/Koi culture and reduced feeding strategies in aquaculture.
3. The research outcomes, should they prove effective will also be disseminated through production of an extension factsheet in the local language for wider outreach to farmers, extension agencies of the government, and NGOs.
4. As part of Investigation 5, an estimate of 60 local farmers, extension agents or other stakeholders will receive training on the results and benefits of these studies through two workshops held in the greater Mymensingh area of Bangladesh.

Schedule

July 2016 to February 2017: Conduct Experiment 1 pond trial to evaluate reduced-feeding strategies for koi-carp polyculture; water quality analyses, data tabulation and analyses, write-up of initial report

March 2017 to November 2017: Experiment 2 pond trial evaluating effect of combining Shing at different stocking densities in Koi-carp polyculture; laboratory analyses, data tabulation and analyses, write-up of report.

December 2017 to February 2018: Generate an extension factsheet, write up Final Technical Report for the work and prepare a manuscript.

DISSEMINATION OF AQUAFISH INNOVATION LAB TECHNOLOGIES FOR IMPROVING FOOD PRODUCTION EFFICIENCY AND LIVELIHOODS OF THE PEOPLE OF BANGLADESH

ASIA PROJECT: BANGLADESH
US Project PI: Russell Borski, North Carolina State University
HC Project PI: Shahroz Mahean Haque, Bangladesh Agricultural University

Mitigating Negative Environmental Impacts/Activity/16MNE02NC

Collaborating Institutions and Lead Investigators

Bangladesh Agricultural University (Bangladesh)	Shahroz Mahean Haque Kaniz Fatema Sadika Haque Ashraful Islam
Patuakhali University of Science and Technology (Bangladesh)	Zahid Parvez Sukhan Md. Lokman Ali
Khulna University (Bangladesh)	Khandaker Anisul Huq
Shushilan NGO (Bangladesh)	Sattyananda Biswas
North Carolina State University (USA)	Russell Borski

Objectives

1. Provide workshops to disseminate promising technologies derived from AquaFish Innovation Lab research.
2. Produce brochures or 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.

Significance

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, salt incursion, and extreme weather (e.g., 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, including: 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 the proposed as well as our previous AquaFish Innovation Lab research in Bangladesh to farmers and their communities through a series of workshops and brochures.

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 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.

Workshop topics according to regions where the promising technologies were developed:

Mymensingh

1. Reduced feeding strategies to improve growth and production efficiency and incomes for tilapia farming.
2. Polyculture with major Indian carps combined with reduced feeding enhances total fish production and profitability of farming tilapia.
3. The high value Shing catfish, an air-breathing fish rich in iron, can be grown in semi-intensive pond culture conditions, reducing environmental impacts of intensive practices. Incorporating major Indian carps into Shing culture and reducing feed inputs can enhance income opportunities by 85%.
4. Polyculture of the air-breathing climbing perch (Koi) with carps represents another profitable polyculture technology that can be adopted by farmers. One of the proposed studies will evaluate the feasibility of growing Koi, Shing, and carps together as an additional polyculture technology for enhancing production of high value crops and income for farmers.

Barishal

5. *Pangasius* catfish grow as effectively in brackish waters as in freshwater ponds, providing a new livelihood alternative for farmers impacted by rises in surface water salinities. Incorporating less costly, locally made formulated feeds, provides additional cost benefits and improves returns on investment for culturing *Pangasius*.
6. Incorporation of tilapia and Koi into polyculture with *Pangasius* in brackish (hyposaline) waters could prove more profitable than *Pangasius* monoculture, providing additional income and diversity of fishes for farming in Southern Bangladesh. This, along with establishing whether Koi can be produced in hyposaline brackish waters will be tested in one of the investigations outlined in this proposal.

Khulna

7. Incorporating *Mola*, a small indigenous fish species with high nutrient value, or carps, or both into current prawn-gher farming practices does not adversely impact, but in fact enhances the production of prawn. Farmers in utilizing these practices can consume additional nutrients from domestic consumption of *Mola* while improving production of prawn as a cash crop.
8. We 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. We will take this integrated aquaculture-agriculture system and evaluate the types of fertilizers that might enhance prawn-*Mola*-carp culture while

also assessing if different pond mud treatments can further enhance production of fruity vegetables.

A major purpose of these workshops is not only to inform farmers, but to disseminate the new technologies to other relevant extension organizations and stakeholders. This way the impacts of the research and adoption of improved aquaculture management practices will have a higher probability of continuing should the AquaFish Innovation Lab activities in Bangladesh come to an end.

In the proposed activities, we will provide a series of workshops complemented by production and distribution of leaflets that outline the most promising aquaculture findings to the farming community in three major regions of Bangladesh where much of the research was demonstrated in ponds of local farmers or at university field sites.

Quantified Anticipated Benefits

1. Provide six workshops or training sessions for farmers, government fisheries extension agents, NGOs and other stakeholders to disseminate valuable fish and shellfish culture technologies for improving food production efficiency and sustainability while increasing household incomes and fish consumption, thereby contributing to greater food security in the region.
2. Brochures and leaflets (n = 3-5) will be produced outlining the improved culture technologies, the number dependent on the outcomes of Investigations 1-4. These will be supplemented by those already produced for extension of previous research findings.
3. Approximately 150 individuals will benefit from direct training on ways to improve aquaculture production efficiency, household income and nutrition, and management practices for environmentally sustainable farming.

Research Design and Activity Plan

Location

Workshops will be provided in each of the 3 major regions of Bangladesh where improved aquaculture management practices and new technologies continue to be developed as outlined in the proposal. They include the greater Mymensingh, Khulna, and Barishal regions. The workshops will be held at local universities, *i.e*. Bangladesh Agricultural University, Khulna University, and Patuakhali Science and Technology University) and/or community centers within each area.

Methods

We will work with local university, government, and NGO representatives, extension agents and officials who will attend, promote, and contribute to the outreach activities given to farmers and other stakeholders. This will aid in disseminating information in the best and most accepted methods in these areas. We will develop the materials and presentations and organize the workshops. Brochures or leaflets outlining the improved management practices and aquaculture technologies will be developed and produced by the PIs in the local language and distributed by them, local extension agencies, and NGOs in the relevant areas.

Two 1-day workshops will be presented in each of the three regions of Bangladesh: Khulna, Patuakhali, and Mymensingh (a total of 6 workshops). Presentations will focus on the aquaculture developments that came from research in each of the regions (see below). For each workshop, it is anticipated that members of 30+ farming households will be trained on the new technologies. We will also target and assist women in the farming community on better aquaculture practices, as these are the individuals often garnered to take care of food crops and provide nutrition to their children. We will invite farmers with whom we have worked and who actually benefited from the AquaFish Innovation lab research. This will have the effect of introducing other farmers to the real-world benefits of the new culture technologies, increasing the likelihood that others might 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 will be provided to those who require it. Lunch, snacks, tea, and soft drinks will also be provided for the day's events.

Some of the topics for the workshop extension activities will include:

1. Reduced feeding strategies for tilapia monoculture and polyculture with major Indian carps.
2. Hyposaline culture of *Pangasius* catfish and use of formulated diets to reduce costs and improve production efficiency.
3. Potential for tilapia-Koi-*Pangasius* polyculture in brackish water as a livelihood alternative for coastal farmers impacted by salinity contamination of freshwater farming systems
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.

Trainings and Deliverables

- 3-5 factsheets/leaflets or brochures will be produced for distribution to the farming community, government, and NGO agencies, and other stakeholders that outline the new aquaculture technologies developed by the AquaFish Innovation Lab in Bangladesh. The 3-5 brochures produced will include those produced as deliverables under Investigations 1-4.

- Six workshops or training sessions (listed below) for farmers, government fisheries extension agents, NGOs, and other stakeholders will disseminate valuable fish and shellfish culture technologies for improving food production efficiency and sustainability while increasing household incomes and fish consumption in Bangladesh.

- The workshops will provide training to an estimated 150 individuals from 2-3 villages within each of 3 different regions of Bangladesh, thereby contributing to the opportunities to improve food security and household income and nutrition throughout Bangladesh.

  Six workshops planned on the following topics & locations:
  1. Mymensingh: Topics related to reduced feeding strategies and/or polyculture
  2. Barishal: Topics on *Pangasius* culture in brackish water
  3. Khulna: Topics associated with incorporating Mola polyculture and integrated aquaculture-agriculture systems

Schedule

March 2016 to April 2017: Develop and produce workshop presentations and brochures based on aquaculture technologies previously tested and those being assessed in the first year of the proposal.

May 2017 to December 2017: Conduct workshops in the 3 regions of Bangladesh

January 2018 to February 2018: Write technical report.

APPENDIX I: LITERATURE CITED

Literature Cited, North Carolina State University

Ahmed N., Alam, M.F. and Hasan, M.R. 2010. The economics of sutchi catfish (*Pangasianodon hypophthalmus*) aquaculture under three different farming systems in rural Bangladesh. *Aquaculture Research* 41, 1668-1683.

Ahmed, S. 2011. Urea fertilizer for Bangladesh-challenges and opportunity. *Journal of Chemical and Engineering* 26, 22–26.

Al-Harbi, A.H., Uddin, M.N. 2004. Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* X *Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture* 229, 37-44.

Al-Harbi, A.H., Uddin, N. 2005. Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture* 250, 566-572.

Ali, M., Nicieza, A., Wootton, R.J. 2003. Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries* 4, 147-190.

Ali, H., Haque, M.M. and Belton, B. 2013. Striped Catfish, (Pangasianodon hypophthalmus, Sauvage, 1878) aquaculture in Bangladesh: an overview. *Aquaculture research* 44, 950-965.

Ali, M.L., Wahab, M.A. and Borski, R. 2015. [Online] Development of *Pangasius* Catfish Culture Technology in Hyposaline Waters. *AquaNews*, 30(3): 1, 3. Available at: http://aquafishcrsp.oregonstate.edu/Documents/Uploads/FileManager/AquanewsSummer%202015_FINAL_2.pdf [Accessed on 10 February 2016]

Alim, M. A., Wahab, M. A. & Milstein, A. 2004. Effects of adding different proportions of the small fish punti (Puntiussophore) and *Mola* (*Amblypharyngodon mola*) to a polyculture of large carp. *Aquaculture Research* 35, 124-133.

Apu, N.A. 2014. Farmed fish value chain development in Bangladesh: Situation analysis and trends. WorldFish/ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Ashwell, C.M., Angel, R. 2010. Nutritional genomics: a practical approach by early life conditioning with dietary phosphorus. *Revista Brasileira de Zootecnia* 39, 268-278.

Basak, J.K., Titumir, R.A.M., Alam, K. 2015. Future fertiliser demand and role of organic fertiliser for sustainable rice production in Bangladesh. *Agriculture, Forestry and Fisheries* 4, 200–208.

Belton, B., Karim, M., Thilsted, S., Jahan, K.M., Collis, W., Phillips, M. 2011. Review of Aquaculture and Fish Consumption in Bangladesh. Studies and Reviews 2011-53, the World Fish Center.

Bolivar, R.B. Brown, Jimenez, E.B.J. and Brown, C.L. 2006. Alternate day feeding strategy for Nile tilapia growout in the Philippines: Marginal cost-revenue analysis. *North American Journal of Aquaculture*, 68, 192-197.

Borski, R.J., Bolivar, R.B., Jimenez, E.B.T., Sayco, R.M.V., Arueza, R.L.B., Stark, C.R., Ferket, P.R. 2011. Fishmeal-free diets improve the cost effectiveness of culturing Nile tilapia (*Oreochromis niloticus*, L.) in ponds under an alternate day feeding strategy. CRSP Aquafish Proceedings, Shanghai, China.

Bröer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. *Physiol Rev* 88, 249-286.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez Pena, A., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335- 336.

Chakraborty, B.K., Mirza, M.J.A. 2008. Growth and yield performance of threatened Singi, *Heteropneustes fossilis* (Bloch) under semi intensive aquaculture. *J. Fish. Soc. Taiwan* 35, 117-125.

Chakraborty, B.K., Nur, N.N. 2012. Growth and yield performance of Shingi, *Hetoropneustes fossilis* and Koi, *Anabus testudineus*, in Bangladesh under Semi-intensive culture systems. *Int. J. Agril. Res. Innov. & Tech.* 2, 15-24.

Chotipuntu, P., Avakul, P. 2010. Aquaculture potential of climbing perch, *Anabas testudineus*, in brackish water. *Walailak J. Sci. & Tech*. 7, 15-21.

Chowdhury, M.I., Mahmud A.I., Rahman, A.F.M.A. 2014. Effects of Water Salinity on Feeding Efficiencies, Growth Performances and Survival Rate of Thai Strain Koi, *Anabas testudineus* (Bloch, 1792). *World J. Fish & Mar. Sci*. 6, 479-486.

D'Abramo, L.R., Silva, J.L., Frinsko, M.O. 2009. Sustainable farming of freshwater prawns and the assurance of product quality. Bulletin 1188. Mississippi Agricultural and Forestry Experiment Station. Mississippi State University.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G., Lionetti, P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Acadamy of Sciences USA* 107, 14691-14696.

De Jesus-Ayson E.G.T., Borski. 2012. Ration Reduction, Integrated Multitrophic Aquaculture (Milkfish-Seaweed-Sea Cucumber) and Value-Added Products to Improve Incomes and Reduce the Ecological Footprint of Milkfish Culture in the Philippines, p 320-335. Technical Reports: Investigations 2009-2011, AquaFish Collaborative Research Support Program. Oregon State University. Vol 2. 414 pp

Dennis, M., Riza, A. Wilfredo, C., Ma. Severa Fe, K., Roman, S., Rolando, E., Bernard, C., Jerome, L., Pierre, M., Jean Francois, B., Xavier, R. 2004. Salinity tolerance of Oreochromis niloticus and O. mossambicus F1 hybrids and their successive backcross, In: Proceeding of the Sixth International Symposium on tilapia in Aquaculture (ed. by R. Bolivar, G. Mair & K. Fitzsimmons), pp. 426-438. Bureau of Fisheries and Aquatic Ressources, Manilla, Philippines.

Department of Fisheries. 2012. Fish Week Compendium. *Matshya Shakalon.* Department of Fisheries. Ministry of Fisheries and Livestock.

Department of Fisheries. 2012. Fish Fortnight Compendium 2012. Department of Fisheries, Dhaka, Bangladesh.

Department of Fisheries. 2015. National Fish Week 2015 Compendium (in Bangla). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh. 148 pp.

Dey, M.M., Bose, M.L., Alam, M.F. 2008. Recommendation domains for pond aquaculture. Country case study: Development and status of freshwater aquaculture in Bangladesh. WorldFish Center Studies and Reviews No. 1872. The WorldFish Center, Penang, Malaysia. 73 pp.

Edwards, P., Hossain, M.S. 2010. Bangladesh Seeks Export Markets for Striped Catfish. *Global* Aquaculture Advocate, May-June, 65-68.

Egna, H.S. and C.E. Boyd (eds.). 1997. Dynamics of Pond Aquaculture. CRC Press LLC. Boca Raton, Florida. 471 pp.

El-Sayed, A.F.M. 2006. Tilapia Culture. CABI Publishing, Oxford, U.K. 277 pp.

El-Sayed. A.F.M. 2006. Tilapia culture in salt water: Environmental requirements, nutritional implications and economic potentials. In: Editors; E.C. Suarez, D.R. Marie, M.T. Salazar, M.G.N. Lopez, D.A.V. Cavazos, A.C.P.C.A.G. Ortega, Avances en Nutricion Acuicola VIII. VIII Simposium Internacional de Nutricion Acuicola. 15-17 November. Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico.

Fadrosh, D.W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M., Ravel, J. 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* 2, 6.

Ferket, P.R. 2013. Novel nutritional applications to optimize feed efficiency in turkeys. Proceedings of the 7th Turkey Science and Production Conference 85 pp.
Food and Agriculture Organization of the United Nations. 2013. Species fact sheet: *Oreochromis niloticus*. Available online at: http://www.fao.org/fishery/species/3217
FRG 2012. Fertilizer recommendation guide (FRG), Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka 1215. 274 p.
Guerden, I., Aramendi, M., Zambonino-Infante, J., Panserat, S. 2007. Early feeding of carnivorous rainbow trout (*Oncorhynchus mykiss*) with a hyperglucidic diet during a short period: effect on dietary glucose utilization in juveniles. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 292, R2275-R2283.
Halver, J.E. 1984. Special methods in pond fish husbandry. AkademiaiNyomda, Budapest. 146 pp.
Hasan M., Khan M.M.R., Rahman A. 2007. Some biological aspects of Thai Koi, *Anabus testudineus* (Bloch). *J. of Bangla. Agril. Univ*. 5, 385-392.
Heikkinen, J., Vielma, J., Kemiläinen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Navia-Paldanius, D., von Wright, A. 2006. Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 261, 259-268.
Huang da, W., Sherman, B.T., Lempicki, R.A. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* 4, 44-57.
Hussain, M.G. 2009. A future for the tilapia in Bangladesh. *AQUA Culture Asia-Pacific Magazine* July/August 2009, 38-40.
Jasmine, S., Ahamed, F., Rahman, S.H., Jewel, M.A.S., Hossain, M.Y. 2011. Effects of organic and inorganic fertilizers on the growth performance of carps in earthen ponds through polyculture system. *Our Nature* 9, 16-20.
Javed, M., Hassan, M., Javed, K. 1993. Fish fertilization. 5. Effect of artificial feed on the growth performance of major carps. *Pak. J. Agric. Sci* 30, 7-12.
Kohinoor A.H.M., Jahan D.A., Khan M.M., Hossain M.G. 2011. Induced Breeding of Koi (*Anabus testudineus*) and its culture management. Ed: Director General, Bangladesh Fisheries Research Institute (BFRI), Mymenshingh. (Booklet*: In Bengali)*.
Kunda, M., Azim, M.E., Wahab, M.A., Roos, N., Thilsted, S.H., Dewan, S. 2008. The potential of mixed culture of freshwater prawn (*Macrobrachium rosenbergii*) and small indigenous *Mola* (*Amblypharyngodon Mola*) in rain-fed rotational rice-fish culture systems in Bangladesh. *Aquaculture Res.* 39, 506-517.
Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31, 814-821.
Lightfoot, C., Cruz, C.R.D., Carangal, V.R. 1990. International Research Collaboration in rice-fish research. *NAGA-The ICLARM Quarterly* 13, 12-13.
Lucas, A. 1998. Programming by early nutrition: An experimental approach. *The Journal of Nutrition* 128, 401S-406S.
Marques, T.M., Wall, R., Ross, R.P., Fitzgerald, G.F., Ryan, A., Stanton, C. 2010. Programming infant gut microbiota: influence of dietary and environmental factors. *Current Opinion in Biotechnology* 21, 149-156.
Micronutrients Initiatives/UNICEF. 2004. Vitamin A and mineral deficiency: A global report. Ottawa, Canada.
Mjoun, K., Rosentrater, K.A., Brown, M.L. 2010. TILAPIA: Environmental Biology and Nutritional Requirements. South Dakota Cooperative Extension Service No. FS963-02.
Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods* 5, 621-628.

Munir, S.A.M. 2009. Socio-economic impacts and sustainability of Pangasius (P*angasianodon hypophthalmus*) farming in Trishal Upazila under Mymensingh, Bangladesh. MS thesis, Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK.

Nadirah, M., Munafi, A.B.A., Anuar, K.K., Mohamad, R.Y.R., Najiah, M. 2014. Suitability of water salinity for hatching and survival of newly hatched larvae of climbing perch, *Anabas testudineus*. *Songklanakarin J. Sci. Technol*. 36, 433-437.

Nayak, S.K. 2010. Role of gastrointestinal microbiota in fish. *Aquaculture Research* 41, 1553-1573.

Neogi, M.G. (2012). Salinity intrusion in the coastal region of Bangladesh. The Daily Ittefaq, Date; 25/09/2012, P.20.

New, M.B., Valenti, W.C., Tidwell, J.H., Kutty, M.N. 2010. Freshwater Prawns: Biology and Farming. Wiley-Blackwell, Oxford, United Kingdom. 531pp.

Payne. A.I., Collinson. R.I. 1983. A comparison of the biological characteristics of Sarotherodon niloricus (L.) with those of S. aureus (Steindachner) and other tilapia of the delta and lower Nile. *Aquaculture* 30, 335-351

Picha, M.E., Silverstein, J.T., Borski, R.J. 2006. Discordant regulation of hepatic IGF-I mRNA and circulating IGF-I during compensatory growth in a teleost, the hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). *General and Comparative Physiology* 147, 196-205.

Qin, J., Culver, D. A., Yu, N. 1995. Effect of organic fertilizer on heterotrophs and autotrophs: implications for water quality management. *Aquaculture Research* 26, 911-920.

Roos, N., Wahab, M.A., Mostafa, A.R.H., Thilsted, S.H. 2007. Linking human nutrition and fisheries: Incorporating micronutrient-dense, small indigenous fish species in carp polyculture production in Bangladesh. *Food Nutrition Bull* 28, S280-293.

Saha K.C. & Guha B.C. (1939). Nutritional investigation on Bengal fish. *Indian J. Medical Res.,* 26: 921-927.

Singh M.P. & Goswami U.C. (1989). Studies on age and growth of an air-breathing catfish *Heteropneustes fossilis* (Bloch). J. Inland Fish. Soc. India, 21: 17-24.

Soil Resources Development Institute (SRDI). 2010. Saline Soils of Bangladesh; SRDI, Ministry of Agriculture: Dhaka, Bangladesh.

Stone, E.A., Ayroles, J.F. 2009. Modulated modularity clustering as an exploratory tool for functional genomic inference. *PLoS Genetics* 5, e1000479.

Suresh, A.V., Lin, C.K. 1992. Tilapia culture in saline waters: a review. *Aquaculture* 106, 201-226.

Thilsted, S.H., Roos, N., Hassan, N. 1997. The role of small indigenous fish species in food and nutrition security in Bangladesh.NAGA-The ICLARM Quarterly 20, 82-84; 102.

United Nations Food and Agriculture Organization. 2010. Cultured Aquatic Species Information Programme. *Pangasius hypophthalmus*. FAO Fisheries and Aquaculture Department [online]. Rome. http://www.fao.org/fishery/culturedspecies/Pangasius_hypophthalmus/.

Vadra A. 2012. Seed production and culture management of Shing (*Heteropneustes fossilis*). Ed: Director General, Bangladesh Fisheries Research Institute (BFRI), Mymenshingh. (Booklet*: In Bengal.)*

Vadra A., Sultana N. 2012. Induced Breeding of Shing (*Heteropneustes fossilis*) and its culture management. Fish Culture and Management Technology Guidelines. Ed: Director General, Bangladesh Fisheries Research Institute (BFRI), Mymenshingh. *Pp 7-10 (In Bengali).*

Vagner, M., Zambonino-Infante, J.L., Robin, J.H., Person-Le Ruyet, J. 2007. Is it possible to influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a nutritional conditioning during larval stage? *Aquaculture* 267, 165-174.

Wahab, M.A., Little, D., Verdegem, M., Kabir, S., Manjurul, K. 2001. Fish in the pond and crops in the dyke: an integrated farming system (in Bengali). Extension manual EU-funded Pond Live Project. BAU 8 p.

Wahab, M.A., Thilsted, S.H., Hoq, M.E. 2003. Small indigenous species of fish in Bangladesh: Culture potentials for improved nutrition and livelihood. Proceedings of ENRECA/DANIDA funded workshop held on 30-31 October 2002. Bangladesh Agricultural University (BAU), Mymensingh. 166 pp.

Wahab, M.A., Kunda, M., Azim, M.E., Dewan, S., Thilsted, S.H. 2008. Evaluation of freshwater prawn- small fish culture concurrently with rice in Bangladesh. *Aquaculture Res* 39, 1524-1532.

Wahab, M.A., Kader, A., Milstein, A., Kunda, M. 2011.Manipulation of species combination for enhancing fish production in polyculture systems involving major carps and small indigenous fish species. *Aquaculture* 321, 289-297.

Wahab, M.A., Ahmed-Al-Nahid, S., Ahmed, N., Haque, M.M., Karim, M.M. 2012. Current status and prospect of farming of Giant River prawn *Macrobrachiumrosenbergii* (De Man) in Bangladesh: a review. *Aquaculture Res* 43, 970-983.

Welker, T.L., Lim, C. 2011. Use of probiotics in diets of tilapia. *Journal of Aquaculture Research and Development* S1-014.

West, K.P. 2002. Extent of vitamin A deficiency among pre-school children and women at reproductive age. *J Nutrition* 13, 2857S-2866S.

Literature Cited, University of Connecticut at Avery Point

Abol-Munafi AB, Ambak MA, Ismail P, Bui M (2007) Molecular data from the cytochrome b for the phylogeny of channidae (Channa sp.) in Malaysia. Biotechnology, 6, 22–27.

Adamson EAS, Hurwood DA, Mather PB (2010) A reappraisal of the evolution of Asian snakehead fishes (Pisces, Channidae) using molecular data from multiple genes and fossil calibration. Molecular phylogenetics and evolution, 56, 707–17.

Ahmed, M., Hap, N., Ly, V., and M. Tiongco (1998) Socioeconomic Assessment of Freshwater Capture Fisheries of Cambodia. Report on A Household Survey. Project for Management of Freshwater Capture Fisheries of Cambodia. Department of Fisheries, Cambodia; Danish International Development Assistance (DANIDA); and Mekong River Commission (MRC). Phnom Penh, Cambodia. 185p.

Allendorf FW, Luikart G (2007) Conservation and the Genetics of Populations.

Allison EH, Perry AL, Badjeck MC, Neil Adger W, Brown K., Conway D, Halls AS, Pilling GM, Reynolds JD, Andrew NL, Dulvy NK. 2009. Vulnerability of national economies to the impacts of climate change on fisheries. Fish and Fisheries, 10(2):173-196.

Arockiaraj, A.J., M. Muruganandam, K. Marimuthu and M.A. Haniffa. 1999. Utilization of carbohydrates as a dietary energy source by striped murrel, *Channa striatus* (Bloch) fingerlings. *Acta Zool. Taiwan* 10: 103-111.

ASEAN Food Composition Table, 2000. Institute of Nutrition, Mahidol University, INFOODS Regional Database Center. A Handbook for Nutrition, 157pp.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16, 37–48.

Cambodia Demographic Health Survey in 2014, Phnom Penh, Cambodia

CFDO-IMM, 2005. Cambodia Post-Harvest Fisheries Overview. Community Fisheries Development Office, Department of Fisheries, Ministry of Agriculture, Forestry and Fisheries, Cambodia

Corazon VC Barba and Ma Isabel Z Cabrera, 2008. Recommended Dietary Allowances Harmonization in Southeast Asia. Asia Pac J Clin Nutr 2008; 17 (S2):405-408

Darias, M.J., D. Mazurais, G. Koumoumdouros, C.L. Cahu, J.L. Zambonino-Infante, 2011. Overview of vitamin D and C requirements in fish and their influence on the skeletal system. Aquaculture 315: 49-60.

Duc, P.M., T.N. Tuan, and T.T.T. Hien, 2012. An investigation on pathogen infection to cultured snakehead (*Channa striata*) in An Giang and Dong Thap province. Journal of Science, Can Tho University. 21b: 124-132.

Duc, P.M., T.N. Tuan and K. Hatai, 2013. Pathogenicity of *Aeromonas hydrophila* to snakehead (*Channa striata*) fingerlings. Fisheries pathothology, Japan 48 (2): 48-51

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10, 564–567.

FiA (2009). Fishing for the future: A strategic planning framework (SPF) for fisheries: 2009- 2018

Frankham R (2005) Genetics and extinction. Biological Conservation, 126, 131–140.

Gaither MR, Bowen BW, Bordenave TR et al. (2011) Phylogeography of the reef fish Cephalopholis argus (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. BMC Evolutionary Biology, 11.

Hallerman E (2008) Application of risk analysis to genetic issues in aquaculture. FAO Fisheries and Aquaculture Technical Paper, 47–66.

Halls, A.S., Chheng, P., So, N.; Thuok, N. (2012). Food and Nutrition Security Vulnerability to Mainstream Hydropower Dam Development in Cambodia: Impacts of mainstream dams on fish yield and consumption in Cambodia. Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration, March 2012, 90 pp.

Hap, N., Kandarith K., and Battarai, M. (2006). Assessment of Production Cost of Inland Fisheries in Cambodia. Fisheries Administration (FiA). Phnom Penh, Cambodia.

Hien, T.T.T., T.T. Be, C.M. Lee, D.A. Bengtson, 2015. Development of formulated diets for snakehead (*Channa striata* and *Channa micropeltes*): can phytase and taurine supplementation increase use of soybean meal to replace fish meal? Aquaculture 448: 334-340.

Hien, T.T.T., T.D. Dinh, T.M. Phu and D.A. Bengtson. 2015. Assessment of the trash-fish diet for snakehead aquaculture in Vietnam: species composition and chemical characterization**.** Asian Fisheries Science 28: 165-173.

Hien, T.T.T., N.H.D. Trung, B.M. Tam, V.M.Q. Chau, N.H. Huy, C.M. Lee and D.A. Bengtson. Submitted *a*. Replacing freshwater small-size fish by formulated feed in snakehead (*Channa striata*) aquaculture: experimental and commercial-scale pond trials, with economic analysis. Aquaculture Reports.

Hien, T.T.T., P.M. Duc, T.L.C. Tu, T.M. Phu, D.T.M. Thy, and D.A. Bengtson. Submitted *b*. Growth performance and immune response of snakehead (*Channa striata*) fed soy diets with supplementation of mannan oligosaccharide. Asian Fisheries Science.

Hulsey CD, García-de-León FJ (2013) Introgressive hybridization in a trophically polymorphic cichlid. Ecology and Evolution, 3, 4536–4547.

Hutchings JA, Fraser DJ (2008) The nature of fisheries- and farming-induced evolution. Molecular Ecology, 17, 294–313.

Lahr, Michael L. and Erik Dietzenbacher, eds. Input-Output Analysis: Frontiers and Extensions. Palgrave, 2001.

Laikre L, Schwartz MK, Waples RS, Ryman N (2010) Compromising genetic diversity in the wild: Unmonitored large-scale release of plants and animals. Trends in Ecology and Evolution, 25, 520–529.

Loc, V. T. T., Sinh, L. X. and Bush, S. (2007). Trans-boundary Challenges for Fisheries Policy in the Mekong Delta, Vietnam: Implications for Economic Growth and Food Security. Vietnam. Miller, Ronald E. and Peter D. Blair. Input-Output Analysis: Foundations and Extensions, 2nd edition. Cambridge University Press, 2009.

Mekong River Commission. 2009. Hydropower Sector Review for the Joint Basin Planning Process.

Nazia AK, Suzana M, Azhar H, Nguyen Thuy TT, Siti Azizah MN (2010) No genetic differentiation between geographically isolated populations of Clarias macrocephalus G??nther in Malaysia revealed by sequences of mtDNA Cytochrome b and D-loop gene regions. Journal of Applied Ichthyology, 26, 568–570.

Nelis, H.J., A.P. De Leenheer, G. Merchie, P. Lavens and P. Sorgeloos, 1997. Liquid chromatographic determination of vitamin C in aquatic organisms. J. Chromatorgr. Sci. 35: 337- 341.

Nen Phanna, So Nam, Pheng Seang Hay, Pomeroy Robert (2015). Sustainable snakehead aquaculture development in the Lower Mekong River Basin of Cambodia: Weaning and Grow- out of Striped Snakehead (*Channa striata*) in Cambodia. Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration, September 2015, 22 pp.

Nikolsky GV (1963). The ecology of fishes.Academic Press.London and New York.p. 352.

Pham Minh Duc, So Nam, Tran Thi Thanh Hien, and Pomeroy Robert (2011). Sustainable snakehead aquaculture development in the Lower Mekong River Basin of Cambodia and Vietnam- Part 2: Striped Snakehead Fish Diseases and Water Quality Analysis. Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration, Phnom Penh and Can Tho University, Viet Nam. 22 pp.

Phillips MJ (2002). Freshwater aquaculture in the Lower Mekong Basin. Technical Paper No. 7, Mekong River Commission, Phnom Penh. 62 pp. ISSN: 1683-1486.

Posada, D., 2004. Collapse Ver. 1.2. A Tool for Collapsing Sequences to Haplotypes [Online] Available from: http://darwin.uvigo.es.

PRIAC, 2006. Policy Reform Impact Assessment, Cambodia. Impacts of the fisheries policy reforms in Kampong Thom and Prey Veng provinces. Community Fisheries Development Office of the Department of Fisheries, Cambodia

Rab, M. A., Hap, N., Ahmed, M., Keang, S. and Viner, K. (2006). Socioeconomics and values of resources in Great Lake-Tonle Sap and Mekong-Bassac area: Results from a sample survey in Kampong Chhnang, Siem Reap and Kandal provinces. Phnom Penh, Cambodia.

Recommended Energy and Nutrition Intakes for Philippines (RENI), 2002. Food and Nutrition Research Institute, Department of Science and Technology. Nutrition Book. Reprinted March 2017.

Roos N., Thorseng H, Chamnan C, Larsen L, Gondolf UH, Thilsted SH. (2007). Iron content in common Cambodian fish species: perspectives for intake in poor, rural households.

Roos, N., et al., 2006.Freshwater Fish as a Dietary Source of Vitamin A in Cambodia. The Journal of Food Chemistry

Roos, N., et al., 2003. Small Indigenous fish species in Bangladesh: Contribute to Vitamin A, Calcium and Iron Intakes. The Journal of Nutrition

Samantaray, K. and S.S. Mohanty. 1997. Interactions of dietary levels of protein and energy on fingerling snakehead, Channa striata. Aquaculture 156: 245-253.

Sarma, K., A.K Pal, N.P. Sahu, S. Ayyappan and K. Baruah, 2009. Dietary high protein and vitamin C mitigates endosulfan toxicity in the spottedmurrel, *Channa punctatus* (Bloch, 1793). Science of the Total Environment 407: 3668-3673.

Sinh LX, Navy H, Pomeroy RS (2012) Value Chain of Snakehead Fish in the Lower Mekong Basin of Cambodia and Vietnam.

So Nam Eng Tong, SouenNorng and Kent Hortle (2005). Use of freshwater low value fish for aquaculture development in the Cambodia's Mekong basin. Consultancy report for Mekong River Commission – Assessment of Mekong Capture Fisheries Project. Inland Fisheries Research and Development Institute, Department of Fisheries, Phnom Penh, Cambodia.

So Nam and Haing L (2006). A Review of Freshwater Fish Seed Resources in Cambodia. A consultancy report for FAO and NACA. Inland Fisheries Research and Development Institute, Department of Fisheries, Phnom Penh, Cambodia.

So Nam, MAES Gregory, Volckaert Filip (2006). Intra-annual genetic variation in the downstream larval drift of sutchi catfish (Pangasianodon hypophthalmus) in the Mekong River. Biological Journal of the Linnean Society 89: 719–728.

So Nam, Lenh SV, Kura Y (2007). Study of the catch and market chain of low value fish along Tonle Sap River, Cambodia- Implications for management of their fisheries (A preliminary study). Inland Fisheries Research and Development Institute, Phnom Penh.

So Nam (2009). Snakehead culture in the Mekong Delta of Vietnam. Inland Fisheries Research and Development Institute, Phnom Penh.

So Nam, LengSyVann, PrumSomany, Le XuanSinh, and Pomeroy Robert (2009). Assessment Of Diversity And Bioecological Characteristics Of Low Value/Small-Size Fish. Inland Fisheries Research and Development Institute, Phnom Penh. 19 pp.

So Nam, Sam Narith, Dr. Bui Minh Tam, Dr. Tran Thi Thanh Hien, and P. Robert (2011). Sustainable snakehead aquaculture development in the Lower Mekong River Basin of Cambodia and Vietnam-Part 1: Breeding and Weaning of striped snakehead (*Channa striata*) in Cambodia. Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration, September 2011, 57 pp.

So Nam and Sam Narith (2011). Sustainable snakehead aquaculture development in Cambodia. Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration.

So Nam, Souvanny Phommakone, Ly Vuthy, Theerawat Samphawamana, Nguyen Hai Son, Malasri Khumsri, Ngor Peng Bun, Kong Sovanara, Peter Degen and Peter Starr (2015).

Sousa-Santos C, Gante HF, Robalo J et al. (2014) Evolutionary history and population genetics of a cyprinid fish (Iberochondrostoma olisiponensis) endangered by introgression from a more abundant relative. Conservation Genetics, 15, 665–677.

Sriskanthan, G. &Funge-Smith, S. J. (2011).The potential impact of climate change on fisheries and aquaculture in the Asian region. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30, 2725–2729.

Tave D (1999) Inbreeding and broodstock management. FAO fisheries technical paper 392.

Ten Raa, Thijs. The Economics of Input-Output Analysis. Cambridge University Press, 2005.

Touch Bunthang, So Nam, Chheng Phen, Pos Chhantana, En Net, and Robert Pomeroy, 2015. Food and Nutritional Consumption Survey: Women and Preschool-Age Children in Cambodia. IFREDI, Phnom Penh.

Touch Bunthang, Chheng Phen, So Nam, Wilma Hurdata, 2011, Baseline Assessment of Diet and Nutrition in Cambodia, Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration. 122 pp

Touch Bunthang, Chheng Phen, So Nam, Wilma Hurdata, Nao Thuok (2012). Food and Nutrition Security Vulnerability to Mainstream Hydropower Dam Development in Cambodia: Baseline Assessment of Diet and Nutrition in Cambodia in 2011. Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration, July 2012, 123 pp.

Tran Thi Thanh Hien and Bengtson D (2009). Alternative Feeds for Freshwater Aquaculture Species. Final Technical Report of USAID Grant No.: EPP-A-00-06-00012-00. Can Though University, Vietnam and University of Rhode Island, USA.

Tran Thi Thanh Hien and Bengtson D (2011). Alternative Feeds for Freshwater Aquaculture Species. Final Technical Report of AquaFish CRSP USAID Grant No.: EPP-A-00-06-00012-00. Can Though University, Vietnam and University of Rhode Island, USA.

Ward, D., D.A. Bengtson, C.M. Lee and M. Gomez-Chiarri, 2016. Incorporation of soybean products in summer flounder (*Paralichthys dentatus*) feed: effects on growth and survival to bacterial challenge. Aquaculture 452: 395-401.

Watterson, G.A., 1975. On the number of segregating sites in genetic models without recombination. Theor. Popul. Biol. 7, 256e276

WorldFish Center. 2007. Fisheries and aquaculture can provide solutions to cope with climate change. Issues brief. WorldFish Center, Penang, Malaysia.

Literature Cited, Purdue University

Abdulai, A. and Aubert, D. (2004). A Cross-Section of Household Demand for Food and Nutrients in Tanzania. Agricultural Economics 31, 67-79.

Abebaw S, P. Janekarnkij and V. Wangwacharakul, (2011). Dimensions of Food Insecurity and Adoption of Soil Conservation Technology in Rural Areas of Gursum District, Eastern Ethiopia. Kasetsart 32: 308–18.

ADB (2005). An Evaluation of Small-Scale Freshwater Rural Aquaculture Development for Poverty Reduction. Operations Evaluation Department, Asian Development Bank, Asian Development Bank Publications,Manila,Philippines.

Afuang W., Siddhuraju P. and Becker K. (2003). Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (Moringa oleifera Lam.) leaves on growth performance and feed utilization in Nile tilapia (Oreochromis niloticus L.)

Aheto, D.W., Asare, N.K., Quaynor, B., Tenkorang, E.Y., Asare, C., & Okyere, I. (2012). Profitability of Small-Scale Fisheries in Elmina, Ghana. *Sustainability*, 4: 2785-2794.

Aiga, H., Matsuokad, S., Kuroiwab, C., and Yamamotoe, S. (2009). Malnutrition among children in rural Malawian fish-farming households. Transactions of Royal Society of Tropical Medicine and Hygiene 103:827-33.

Ally, T. (2015) Evaluation of Invertebrates as Protein Source in Nile tilapia (Oreochromis niloticus) Diets. MSc. dissertation, Sokoine University of Agriculture, Morogoro, Tanzania.

Amador, K., Bannerman, P., Quartey, R. & Ashong, R. (2006). Ghana Canoe Frame Survey. Information, Report Number 34. Marine Fisheries Research Division. Ministry of Fisheries. Ghana. 43p.

American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Environment Federation (WEF). (2012). Standard Methods for the Examination of Water and Wastewater, 22nd edition. APHA, Washington, DC.

Ansah, Y. B., and E. A. Frimpong. (2015). Impact of adoption of BMPs on social welfare: a case study of commercial floating feeds for pond culture of tilapia in Ghana. *Cogent Food and Agriculture* 1:1048579

Antwi, V. (2006). “Sustainable Impact Assessment of Proposed WTO negotiations: the Fisheries Sector Country Case Study: Ghana.” Unpublished. Available at: http://trade.ec.europa.eu/doclib/docs/2006/may/tradoc_128851.pdf

Asamoah, E.K., F.K.E. Nunoo, Y.B. Osei-Asare, S. Addo, and U.R. Sumaila. (2012). A Production Function Analysis of Pond Aquaculture In Southern Ghana. Aquaculture Economics & Management. 16(3) :183-201.

Atta-Mills, J., Alder, J. & Sumaila, R. (2004). The Decline of a Regional Fishing Nation: The Case of Ghana and West Africa. *Natural Resources Forum*, 28: 13–21.

Bank of Ghana. (2008). The Fishing Sub-Sector and Ghana’s Economy, Research Department, Bank of Ghana, September 2008, ISBN: 0855-658X

Bazil J. (2014) Evaluation of Plant and Animal Products/By Products as Alternative Protein Sources to Fish Meal in Tilapia Diets

Bell, J.D., Kronen, M., Vunisea, A., Nash, W.J., Keeble, G., Demmke, A., Pontifex, S. and Andréfouét, S. (2009) Planning the use of fish for food security in the Pacific. Mar. Policy, 33: 64–76.

Belton, B., van Assledonk, I., Thilsted, S. (2013) Faltering Fisheries and Ascendant Aquaculture: Implications for Food and Nutrition Security in Bangladesh. Food Policy.

Béné, C. (2003). When Fishery Rhymes with Poverty, a First Step beyond the Old Paradigm on Poverty in Small-Scale Fisheries. *World Development*, 36(1): 945-975.

Biederlack, L. and Rivers, J. (2009) Comprehensive Food Security & Vulnerability Analysis (CFSVA) Ghramme, VAM Food Security Analysis. United Nations World Food Programme, Rome.

Braimah, L. I. (2003). Recent development in the fisheries of the Volta Lake (Ghana). In: RRM. Cruz and F.C. Roe st (Eds). Current status of fisheries and fish stocks of four largest African resource. CIFA Tech Paper 30, 111-134

Chenyambuga, S.W., A. Mwandya, H.A. Lamtane, & N.A. Madalla (2014). Productivity and marketing of Nile tilapia (Oreochromis niloticus) cultured in ponds of small-scale farmers in Mvomero and Mbarali districts, Tanzania. Livestock Research for Rural Development. Volume 26, Article, 43.

Choudhury, N. (2011). Dietary Diversity: Measurement and Interpretation. Training Course on Assessment of Nutritional Status.

Crentsil, C., and F.L. Essilfie. (2014). Measurement of technical efficiency of smallholder fish production in Ghana: A stochastic frontier approach. Journal of Development and Agricultural Economics, 6(5): 203-211.

Cunningham, L. (2005). Assessing the Contribution of Aquaculture of Food Security: A Survey of Methodologies. Food and Agriculture Organization of the United Nations. Rome.

Das, S. K and Jana, B. B. (1996) Pond fertilization through inorganic sources: an overview. Indian J. Fish., 43(2): 137-155

De Silva, S.S. and Davy, E.B. (2010). “Aquaculture success in Asia: Contributing to sustained development and poverty alleviation” In: De Silva, S. and Davy, F.B (eds) Success stories in Asian aquaculture. Springer, International Development Research Centre pp 1-14.

Dey, M. M., Rab, M. A., Paraguas, F.J., Piumsombun, S., Ramachandra, B., Alam, M. F., & Mahfuzuddin, A. (2005). Fish consumption and food security: a disaggregated analysis by Types of Fish and Classes of Consumers in Selected Asian Countries. *Aquaculture Economics and Management*, 9 (1/2): 89 - 111.

Diana, J. S. (2012). Some principles of pond fertilization for Nile tilapia using organic and inorganic inputs. Aquaculture Pond Fertilization: Impacts of Nutrient Input on Production, 1st Edition, Edited by Charles C. Mischke. John Wiley and Sons, Inc., Hoboken. http://dx. doi. org/10.1002/9781118329443. ch12.

Diana, J. S., Lin C. K. and Jaiyen K. (1994). Supplemental feeding of tilapia in fertilized ponds. Journal of the World Aquaculture Society 25:497-506.

Egna, H.S., N. Brown, and M. Leslie (editors). (1987). Pond Dynamics/Aquaculture Collaborative Research Data Reports, Volume 1, General Reference: Site Descriptions, Materials and Methods for the Global Experiment. PD/A CRSP, Oregon State University, Corvallis, Oregon, 84 pp.

El-Saidy D.M.S.D. and Gaber M.M.A. (2002). Complete Replacement of Fish Meal by Soybean Meal with Dietary L-Lysine Supplementation for Nile Tilapia Oreochromis niloticus (L.) Fingerlings. *Journal of the World Aquaculture Society* 33 (3):297-306.

El-Sayed, A.F.M. (1999). Alternative dietary protein sources for farmed tilapia, Oreochromis spp. *Aquaculture* 179 (1-4):149-168.

Engle, C. R., and I. Neira. (2005). Tilapia farm business management and economics: a training manual. AquaFish CRSP, Corvallis, Oregon.

Essuman, K.F. (1992). “A Study on Processing, Marketing and Consumption of Cured Fish in West Africa: Fermented Fish in Africa.” FAO Fisheries Technical Report. No: 329.

FAO. (2002). The State of Food Insecurity in the World 2001. Rome.

FAO (2005) Cultured Aquatic Species Information Programme. Oreochromis niloticus. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (2007). The State of World Fisheries and Aquaculture (SOFIA) 2006. World review of fisheries and aquaculture, Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2007) National Fishery Sector Overview: The United Republic of Tanzania. Available at ftp://ftp.fao.org/FI/DOCUMENT/fcp/en/FI_CP_TZ.pdf.

FAO (2014). The State of Fisheries and Aquaculture - 2014. Rome, Italy: Food and Agriculture Organisation of the United Nations.

FAO. (2014). The State of World Fisheries and Aquaculture 2014. Rome

Fitzsimmons, K. (2013). Latest trends in Tilapia production and market worldwide. World Aquaculture Society, Rio de Janeiro.

Frimpong, E. A., and S. E. Lochmann. (2006). An evaluation of the effect of treatments for pond water reuse on zooplankton populations. *North American Journal of Aquaculture* 68:103-109.

Gomna, A. and Rana, K. (2007) Inter-household and intra-household patterns of fish and meat consumption in fishing communities in two states in Nigeria. Br. J. Nutr., 97: 145–152.

Hailu, M. (2012). Causes of Household Food Insecurity in Rural Boset Woreda: Causes, Extent and Coping Mechanisms to Food Insecurity. Germany: Lap Lambert Academic Publishing.

Harvey, D. US Seafood market Shifts to Aquaculture. http://ers.usda.gov/amber-waves/2004-april/us-seafood-market-shifts-to-aquaculture.aspx#.V20-jTXxiSo. Accessed, June 2016.

Hasan, M.R. & New, M.B., eds. (2013). On-farm feeding and feed management in aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO. 67

Hortle, K. (2007) Consumption and the yield of fish and other aquatic animals from the Lower Mekong Basin. MRC Technical Paper No.16, Vientiane.

Jahan, K. M-e., Ahmed, M. and Beltom, B. (2010). "The impacts of aquaculture development on food security: lessons from Bangladesh". *Aquaculture Research*, 41:481-495.

Kaliba AR, K.O. Osewe, E.M. Senkondo, B.V. Mnembuka and K.K. Quagrainie. 2006. Economic Analysis of Nile Tilapia (Oreochromis niloticus) production in Tanzania. Journal of the World Aquaculture Society. 37(4): 464–473.

Karim, M. (2006). The Livelihood Impacts of Fish Ponds Integrated Within Farming Systems in Mymensingh District, Bangladesh. Ph.D. Thesis, University of Stirling.

Kawarazuka, N. (2010). The Contribution of Fish Intake Aquaculture, and Small-Scale Fisheries to Improving Food and Nutrition Security: A Literature Review. Working Paper.

Kennedy, G., A. Berardo, C. Papavero, P. Horjus, T. Ballard, MC. Dop, J. Delbaere & D. Brower. (2010). Proxy Measures of Household Food Consumption for Food Security Assessment and Surveillance: Comparison of eth Household Dietary Diversity and Food Consumption Scores. *Public Health Nutrition* 13 (12): 2010-2018.

Kent, G. (1987) Fish, Food and Hunger: The Potential of Fisheries for Alleviating Malnutrition West View Press, Colorado, USA (1987).

Kitojo, O. D. (2013).Assessment of feeding strategies based on Moringa oleifera leaf meal and Sunflower seed cake as sources of protein in diets of Nile tilapia (Oreochromis niloticus). MSc. dissertation, Sokoine University of Agriculture, Morogoro, Tanzania.

Lazard, J., Baruthio, A., Mathe, S., Rey-Valette, H., Chia, E., Clement, O., Aubin, J., Morissens, P., Mikolasek, O., Legendre, M., Levang, P., Blancheton, J. and Rene, F. (2010). "Aquaculture system diversity and sustainable development: fish farms and their representation". *Aquatic Living Resources*, 23: 187-198.

Ludwig, G.M., Perschbacher, P. W., and Edziyie, R. (2010). The effect of the dye Aquashade on waterquality, phytoplankton, zooplankton, and sunshine bass fingerling production in fertilized culture ponds. *Journal of World Aquaculture Society*, 41(S1):40-48.

Maxwell, D., Watkins, B., Wheeler, R., & Collins, G. (2003). The coping strategies index: A tool for rapidly measuring food security and the impact of food aid programs in emergencies. Nairobi, Kenya: CARE and World Food Programme.

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 Hεn Mpoano Initiative, Ghana. 57pp. Available at: http://www.worldfishcenter.org/resource_centre/WF_3450.pdf

Ministry of Fisheries and Aquaculture Development – MFAD, (2013). 2012 Annual Report, Accra. Ghana.

Musiba, M.J., G.W. Ngupula, B.B. Kashindye, M. Elison, A.P. Shoko, J. Ndikumana, & E.F.B. Katunzi. (2014). Performance of locally formulated feeds for rearing of African catfish in Tanzania. African Crop Science Journal. 22, 979-986.

National Oceanic and Atmospheric Agency (NOAA) 2015. Marine Aquaculture Strategic Plan FY 2016 - 2020. Seaweb food Summit, Washington DC.

Obirikorang, K. A., Amisah, S., Fialor, S. C., & Skov, P. V. (2015). Effects of dietary inclusions of oilseed meals on physical characteristics and feed intake of diets for the Nile Tilapia, Oreochromis niloticus. *Aquaculture Reports*, 1, 43-49.
Omoyinmi G.A.K. and Olaoye O.J. (2012). Growth Performance of Nile Tilapia-Oreochromis niloticus Fed Diets Containing Different Sources of Animal Protein. Libyan Agriculture Research Center Journal International 3 (1): 18 - 23.
Pant, J., Demaine, H. and Edward, P. (2004). "Assessment of the aquaculture subsystem in integrated agriculture-aquaculture systems in Northeast Thailand". *Aquaculture Research*, 35: 289-298.
Pond Dynamics / Aquaculture Collaborative Research Program (PD/A CRSP). 2000. Handbook of Analytical Methods.3rd Printing. Oregon State University. February 2000. Available at: http://aquafishcrsp.oregonstate.edu/Documents/Uploads/FileManager/Handbook_of_Analytical_Methods.pdf
Pond Dynamics / Aquaculture Collaborative Research Program (PD/A CRSP). 1987. General Reference: Site Descriptions, Materials and Methods for the Global Experiment. Vol. 1. Edited by HS. Egna, N. Brown, and M. Leslie. Oregon State University, August 1987. Available at: http://aquafishcrsp.oregonstate.edu/Documents/Uploads/FileManager/Pond%20Dynamics:Aquaculture%20Collaborative%20Research%20Data%20Reports_Volume%201.pdf
Phanna N., Nam S., Ramara O. and Pomeroy R. (2014). Experimental pond unit assessment in Cambodia. In: AquaFish Collaborative Research Support Program. Goetting, K., Price, C., and Egna, H., (eds). AquaFish CRSP Technical Reports: Investigations 2011 – 2013. AquaFish Innovation Lab, Oregon State University. Corvallis, Oregon. p 70 - 93.
Shigulu, H, J. (2011). Effect of Substituting Sunflower Seed cake and Soybean meal with Moringa Leaf Meal as Protein Supplement on Growth Perfomance of Nile tilapia (Oreochromis niloticus). MSc. dissertation, Sokoine University of Agriculture, Morogoro, Tanzania.
Shoko, A.P, A. Getabu, G. Mwanyuli, and Y.D. Mgaya. 2011. Growth performance, yields and economic benefits of Nile tilapia (*Oreochromis niloticus*) and Kales (*Brassica oleracea*) cultured under vegetable-fish culture integration. Tanzania Journal of Science. 37: 37 - 48.
Swindale, A., & Bilinsky, P. (2006). Household dietary diversity score (HDDS) for measurement of household food access: indicator guide. Washington, DC: Food and Nutrition Technical Assistance Project (FANTA), Academy for Educational Development, USAID.
Thilsted, S.H., D. James, J. Toppe, R. Subasinghe and I. Karunasagar. (2014). Maximizing the Contribution of Fish to Human Nutrition. ICN2 Second International Conference on Nutrition.
Wahab Md. A., Rahaman S.M.B., Milstein A., Daniels H.V. and Borski R.J. (2014). Experimental pond unit assessment in Cambodia. In: AquaFish Collaborative Research Support Program. Goetting, K., Price, C., and Egna, H., (eds). AquaFish CRSP Technical Reports: Investigations 2011 – 2013. AquaFish Innovation Lab, Oregon State University. Corvallis, Oregon. p 1 - 37.
Watanabe W. O., Losordo T. M., Fitzsimmons, K & F.Hanley (2002). Tilapia Production Systems in the Americas: Technological Advances, Trends, and Challenges, Reviews in Fisheries Science, 10:3-4, 465-498.
Wijkstrom, U. N. and N. J. MacPherson. (1990). A cost benefit analysis of culture based fisheries development in small dams and dugouts. Field work paper 1: The economics of culture based fisheries. Field Doc. F1: TCP/GHA0051. FAO, Rome, Italy.
World Bank (2006) Aquaculture: Changing the Face of the Waters: Meeting the Promise and Challenge of Sustainable Aquaculture. World Bank, Washington, DC (2006).
World Food Programme (WFP). (2008). Measures of Food Consumption – Harmonizing Methodologies. Rome. Interagency Workshop Report.
WorldFish Center, (2009). Fish Supply and Food Security for Africa. Available at www.thefishsite.com/articles/1946/aquaculture-and-food-security-poverty-alleviation-and-nutrition-in-ghana.
World Food Programme (WFP). (2009). Emergency Food Security Assessment Manual. Rome.
WorldFish Centre (2011) Aquaculture, Fisheries, Poverty and Food Security. Working Paper 2011-65.

Literature Cited, University of Michigan

Azim, M.E., M.C.J. Verdegem, H. Khatoon, M.A Wahab, A.A van Dam, and M.C.M. Beveridge. 2002. A comparison of fertilization, feeding and three periphyton substrates for increasing fish production in freshwater pond aquaculture in Bangladesh. Aquaculture 212:227-243.

Bhandari, M.P., R. Jaiswal, N.P. Pandit, R.N. Mishra, M.K. Shrestha, and J.S. Diana. 2016. Demonstrating the value of tilapia and sahar production in polyculture ponds using government farm and on-farm trials. AquaFish Innovation Lab, Tenth Annual Report, in press.

Bhujel, R.C., M.K. Shrestha, J. Pant, and S. Buranrom. 2008. Ethnic women in aquaculture in Nepal. Development 51:259-264.

Bista, J.D., T.B. Gurung, S.K. Wagle, and A.K. Rai. 2008. Present status and prospects of sahar (*Tor putitora*) fisheries in Nepal. Journal of the Institute of Agriculture and Animal Sciences 29:1-11.

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

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

Diana, J.S. 2012. Some principles of pond fertilization for Nile tilapia using organic and inorganic inputs. *In* C.C. Mischke (ed.). Pond Fertilization: Impacts of Nutrient Input on Aquaculture Production. John Wiley and Sons, Inc., New York. pp. 163-177.

Diana, J.S., H.S. Egna, T. Chopin, M.S. Peterson, L. Cao, R. Pomeroy, M. Verdegem, W.T. Slack, M.G. Bondad-Reantaso, and F. Cabello. 2013. Responsible aquaculture in 2050: Valuing local conditions and human innovations will be key to success. BioScience 63:255-262.

Diana, J.S., N.P. Pandit, and M.K. Shrestha. 2016. Household fish ponds in Nepal: their constraints determined by value chain analysis. AquaFish Innovation Lab, Tenth Annual Report, in press.

DoFD (Directorate of Fisheries Development). 2012. Annual report 2011-2012. Central Fisheries Building, Balaju, Kathmandu, Nepal.

Egna, H., L. Reifke, and N. Gitonga. 2012. Improving gender equity in aquaculture education and training: 30 years of experiences in the Pond Dynamics/Aquaculture, Aquaculture, and AquaFish Collaborative Research Support Programs. Asian Fisheries Science 25S:119-128.

FAO (Food and Agriculture Organization). 1998. Women feed the world. Prepared for World Food Day, 16 October 1998. Rome, Italy. 1 p.

FAO (Food and Agriculture Organization). 2013. National Aquaculture Sector Overview Nepal. National Aquaculture Sector Overview Fact Sheets. Text by Pradhan, G. *In*: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 10 October 2005. [Cited 30 May 2013]. http://www.fao.org/fishery/countrysector/naso_nepal/en

GoN (Government of Nepal). 2000. Fisheries perspective plan. Submitted to Inland Aquaculture and Fisheries Development Division. Agricultural Projects Services Centre, Kathmandu, Nepal.

Gurung, T.B., A.K. Rai, P.L. Joshi, A. Nepal, A. Baidhya, J. Bista and S.R. Basnet. 2001. Breeding of pond reared golden mahseer (*Tor putitora*) in Pokhara, Nepal. Coldwater Fish Species in the Trans-Himalayan Region, (10-14 July, 2001), Kathmandu, Nepal.

IUCN. 2016. The IUCN red list of threatened species. Accessed on 26 July 2016 at http://www.iucnredlist.org/details/166645/0.

Jaiswal, R. 2012. Integration of tilapia (*Oreochromis niloticus*) and sahar (*Tor putitora*) in carp polyculture system. Master's thesis. Institute of Agriculture and Animal Science, Nepal.

Jha, D.K., N.P. Pandit, I.S. Mahato, M.K. Shrestha, and J.S. Diana. 2016a. Establishing school ponds for fish farming and education to improve health and nutrition of women and children in rural Nepal. AquaFish Innovation Lab, Tenth Annual Report, in press.

Jha, S.K., J.D. Bista, M.K. Shrestha, and J.S. Diana. 2016b. Reproduction and seed production of sahar (*Tor putitora*) in Chitwan, Nepal. AquaFish Innovation Lab, Tenth Annual Report, in press.

Joshi, P.L., T.B. Gurung, S.R. Basnyat, and A.P. Nepal. 2002. Domestication of wild golden mahaseer and hatchery operation. *In*: T. Pert and D.B. Swar (eds.) Cold Water Fisheries in the Trans-Himalayan Countries. FAO Technical Paper No. 431, Rome, pp. 173-178.

Keeler, B., J.S. Diana, N.P. Pandit, and M.K. Shrestha. 2016. Introduction of two small indigenous species to improve sustainability in typical polyculture systems in Nepal. AquaFish Innovation Lab, Tenth Annual Report, in press.

Kloeblen, S. 2011. The role of women in small scale aquaculture in Nepal. Ecological Aquaculture Studies and Reviews, University of Rhode Island, Kingston, R.I.

Little, D.C. 1989. An evaluation of strategies for production of Nile tilapia (*Oreochromis niloticus L.*) fry suitable for hormonal treatment. Ph.D. dissertation. Institute of Aquaculture, University of Stirling, Scotland.

Little, D.C., M. Skladany, and R. Rode. 1987. Small-scale hatcheries in north-east Thailand. Aquaculture and Fisheries Management 18:15-31.

Little, D.C., and P. Edwards. 2004. Impact of nutrition and season on pond culture performance of mono-sex and mixed-sex Nile tilapia (*Oreochromis niloticus*). Aquaculture 232:279–292

Mandal J.K., and M.K. Shrestha. 2001. Effect of feed supplementation on growth and production of Nile tilapia in mixed size culture system. Journal of the Institute of Agriculture and Animal Science 21-22:141-149.

NARC (Nepal Agricultural Research Council). 2010. Meeting Nepal's food and nutrition security goals through agricultural science and technology. *In:* NARC's Strategic Vision for Agricultural Research (2011-2030). Nepal Agricultural Research Council, Kathmandu, Nepal.

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

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

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

Rai, S., M.K. Shrestha, and J.S. Diana. 2016. Production of periphyton to enhance yield in polyculture ponds with carps and small indigenous species. AquaFish Innovation Lab, Tenth Annual Report, in press.

Rai, A.K. 2008. Status of sahar (*Tor putitora*) domestication and its development in the Himalayan Region of Nepal. Aquaculture Asia Magazine, Jan-Mar 2008: 26-32.

Rai S., M.K. Shrestha, D.K. Jha, and D. Acharya. 2007. Growth performance of Sahar and mixed-sex Nile tilapia in monoculture and co-culture system in Chitwan. *In*: R.B. Thapa and M.D. Sharma (eds.), IAAS Research Advances, Volume 1. Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal, pp. 187-193.

Rai, S., and Y. Yi. 2012. Nibbling frequency of carps in Periphyton-Based Aquaculture Systems with and without Supplemental Feed. Israeli Journal of Aquaculture 64:818-822.

Rai, S., Y. Yi, M.A. Wahab, A. Bart, and J.S. Diana. 2008. Comparison of rice straw and bamboo sticks substrates in periphyton-based carp polyculture systems. Aquaculture Research 39:464-473.

Rajbanshi, K.G. 2001. Zoo-geographical distribution and the status of cold water fishes of Nepal. Symposium on coldwater fishes in the trans Himalayan region, 10-14 July, Kathmandu, Nepal.

Rana, P.J.B., and K.G. Rajbanshi. 1976. National Plan for development of aquaculture in Nepal. *In*: Aquaculture Planning in Asia. Report of the Regional Workshop on Aquaculture Planning in Asia, Bangkok, Thailand, 1-17 October 1975. FAO, Rome.

Ranjan, R., B. Xavier, B. Dash, L.L. Edward, R.D. Suresh, and P.S. Kumar. 2015. Efficacy of 17 a-methyl testosterone and letrozole on sex reversal of protogynous grouper, *Epinephelus tauvina* (Forskal, 1775) and spawning performance of sex-reversed males. Aquaculture Research 46:2065–2072.

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

Shrestha, T.K. 1997. The mahseer in the rivers of Nepal disrupted by dams and ranching strategies. Bimala Shrestha, Kathmandu, Nepal, pp. 259.

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

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

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

Shrestha, M.K., J. Pant, R.C. Bhujel. 2012. Small-scale aquaculture development model for rural Nepal. *In*: M.K. Shrestha and J. Pant (eds.) Small-scale Aquaculture for Rural Livelihoods: Proceedings of the National Symposium on Small-scale Aquaculture for Increasing Resilience of Rural Livelihoods in Nepal. Institute of Agriculture and Animal Science, Tribhuvan University, Rampur, Chitwan, Nepal, and The WorldFish Center, Penang, Malaysia, pp. 71-75.

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

Tain, F.H., and J.S. Diana. 2007. Impacts of extension practice: Lessons from small farm-based aquaculture of Nile tilapia in Northeastern Thailand. Society and Natural Resources 20:583-595.

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

Yi, Y., J.S. Diana, M.K. Shrestha, and C.K. Lin. 2004. Culture of mixed-sex Nile tilapia with predatory snakehead. *In*: R. Boliver, G. Mair, and K. Fitzsimons (eds.) New Dimension in Farmed tilapia, Proceedings of 6th International Symposium on Tilapia in Aquaculture (ISTA 6), pp. 544-557.

Literature Cited, Auburn University

Adomi, E. E., Ogbomo, M.O., and Inoni, O. E. 2003. Gender factors in crop farmers' access to agricultural information in rural areas of Delta State. Library Review 52 (8): 388 – 393. Aganyira, K. 2005.

Aquaculture: a tool for sustainable development in Uganda: a case study of Kigoowa Catholic women's development association in Kampala district. Retrieved August 14, 2014, from www.divaportal.org/smash/get/diva2:125644/FULLTEXT01.pdf

Aker, Jenny, C. 2011. Dial "A" for agriculture: a review of information and communication technologies for agricultural extension in developing countries. Agricultural Economics 42 (2): 631–647.

Aruho, C., Basiita R, K. Kahwa, D., Bwanika G & Rutaisire J. 2013). Reproductive biology of Bagrus docmak in the Victoria Nile, Uganda. African Journal Aquatic of Sciences, 38(3): 263–271

Asche, F., D.V. Gordon, and R. Hannesson. 2004. Tests for market integration and the law of one price: the market for Whitefish in France. Marine Resource Economics 19:195-210.

Auburn University. 1999. Uganda fish project fisheries investment for sustainable harvest. The International Center for Aquaculture and Aquatic Environments (ICAAE). Retrieved March 3, 2015, from http://www.ag.auburn.edu/fish/international/currentprojects/ugandafish-project-fisheries-investment-for-sustainable-harvest/ugandafishproject-executive-summary/.

Balarin, J. D. 1985. National reviews for aquaculture development in Africa. 10. Uganda. FAO fisheries circular, No. 770.10. FIRI/C770.10. Rome: Food and Agriculture Organization of the United Nations.

Baltzegar, D.A., Reading, B.J., Brune, E.S., and Borski, R.J. 2013. Phylogenetic revision of the claudin gene family. Marine Genomics 11:17-26. Bancroft, J. D & Gamble, M. 2002). Theory and practice of histological techniques, 5th edn. pp 85-107. Edinburgh, Churchill Livingston Publishers, London.

Berlinsky, D.L. & Specker, J.L. 1991). Changes in gonadal hormones during oocyte development in the striped bass, Morone saxatilis. Fish Physiology and Biochemistry 9(1):51-62.

Berry, K. 2016. Simplifying your mobile solution. Article ID: 20160209: 42. Retrieve February 8, 2016 from http://www.quirks.com/articles/2016/20160209.aspx

Bollerslev, T. 1986. Generalized autoregressive conditional heteroscedasticity. Journal of Econometrics, 31(3): 307-327

Bolton, Ruth & Shruti Saxena-Iyer. 2009. Interactive services: a framework, synthesis and research directions. Journal of Interactive Marketing 23: 91–104

Booth, A. J. 1997. On the life history of the lesser gurnard (Scorpaeniformes: Triglidae) inhabiting the Agulhas Bank, South Africa. Journal of Fish Biology, 51: 1155-1173.

Braat L 1986. Multi-objectives modeling of economic ecological Interactions and conflicts, Urije Universitect, Amsterdam.

Bromage, N., Porter, M. & Randall, C. 2001. The environmental regulation of maturation in farmed finfish, with special reference to the role of photoperiod and melatonin. Aquaculture, 197: 63–98.

Bromage, N., Porter, M. & Randall, C. 2001. The environmental regulation of maturation in farmed finfish, with special reference to the role of photoperiod and melatonin. Aquaculture, 197: 63–98.

Buguk, C., Hudson, D., & D. Hanson. 2003. Price volatility in agricultural markets: An examination of U.S. catfish markets. Journal of Agricultural and Resource Economics, 28(1): 86–99.

Burrell, J and Matovu, J. 2008. Livelihoods and the mobile phone in rural Uganda. Washington DC: The Grameen Foundation, USA. Retrieved on June 22, 2015, from http://www.grameenfoundation.applab.org/section/ethnographic-research.

Campbell, A. 2005. Mobile phones for small African farmers. Retrieved January 4, 2015, from http:// smallbiztrends.com/2005/03/mobile-phones-for-small-african.html.

Chapman, R.W., Reading, B.J., and Sullivan, C.V. 2014. Ovary transcriptome profiling via artificial intelligence reveals a transcriptomic fingerprint predicting egg quality in striped bass, Morone saxatilis. PLoS ONE 9(5):e96818. All authors contributed equally to this manuscript.

Chong Chhachhar, A. R, and Hassan, H. S. J. 2013. The use of mobile phone among farmers for agriculture development. International Journal of Scientific Research 2(6): 2277-8179.

Chou, R. 1988. Volatility Persistence and stock valuations: some empirical evidence using GARCH. Journal of Applied Econometrics 3: 279-294.

Connolly K. and T. Trebic. 2010. Optimization of a Backyard Aquaponic Food Production System. Faculty of Agricultural and Environmental Sciences Macdonald Campus, McGill University.

Dahl Roy Endre and Atle Oglend. 2014. Fish price volatility. Marine Resource Economics 29:4. December.

De Silva, S. S. & Anderson, T. A. 1995. Fish nutrition in aquaculture. Chapman and Hall. Landon. pp.319

Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenburg (editors). 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.

eTransformation Africa. 2012. The transformational use of information and communication technologies in Africa. Washington DC: World Bank. Retrieved March 8, 2015, from: www.etransformafrica.org productivity and competitiveness. 8-12 Sept. Indonesia.

Faker, J. C. 2008. Does digital divide or provide? The impact of cell phones on grain markets in Niger. Center for Global Development working paper No. 154. Retrieved October 15, 2015, from SSRN: http://ssrn.com/abstract=1093374.

FAO. 2007. The Internet and rural and agricultural development: an integrated approach. Rome: Food and Agriculture Organization. Retrieved February 5, 2015, from http://www.fao.org/docrep/w6840e/w6840e05.htm.

Feijaoo C. V. 2011. Synergy between wild and commercial bioeconomic modeling of Python farming. Thesis. Imperial College, London

Food and Agriculture Organization (FAO(. 2014. The state of world fisheries and aquaculture. World review of fisheries and aquaculture. pp 243.

Fowler, B. L., & Buonaccorsi, V. P. 2016. Genomic characterization of sex-identification markers in Sebastes carnatus and S. chrysomelas rockfishes. Molecular ecology.

Frediani, K. 2011. High rise food. The Horticulturalist, October, pp.18 – 20.

Gordon, A.K., Kaiser, H. . Britz, P.J. & Hecht, T. 2000. Effect of Feed Type and Age-at-weaning on Growth and Survival of Clownfish Amphiprion percula (Pomacentridae). Aquarium sciences and conservation, 2 (4):215-226.

Government of Kenya (GoK). 2010. Fisheries Annual Statistical Bulletin. Ministry of Agriculture, Livestock and Fisheries Development.

Government of Kenya (GoK). 2012. Fisheries Annual Statistical Bulletin. Ministry of Agriculture, Livestock and Fisheries Development.

Government of Kenya (GoK). 2014. Fisheries Annual Statistical Bulletin. Ministry of Agriculture, Livestock and Fisheries Development. Greenwood, P.H. 1966. The Fishes of Uganda. 2nd ed. The Uganda Society Kampala, pp 1-6.

Guttormsen, A.G. 1999. Forecasting weekly salmon prices: risk management in fish farming. Aquaculture Economics and Management 3 (2): 159-66.

Hasan, M.R., Hecht, T., S.S. De Silva, and A.G.J. Tacon (eds.). 2007. Study and analysis of feeds and fertilizers for sustainable aquaculture development. FAO Fisheries Technical Paper No. 497. 504 pp.

Hodson, R.G. & Sullivan, C.V. 1993. Induced maturation and spawning of domestic and wild striped bass, Morone saxatilis (Walbaum), broodstock with implanted GnRH analogue and injected hCG. Aquaculture Research 24(3):389-398.

Hudson, H. 2006. From Rural Village to Global Village: Telecommunications for Development in the Information Age. Danbury: Lawrence Erlbaum Associates, Incorporated. IBM. 2011. Milwaukee Report, IBM CorporateCitizenshipandCorporateAffairs,32p.

Ilahiane, H. 2007. Impacts of information and communication technologies in agriculture: Farmers and mobile phones in Morocco. Paper presented at the Annual Meetings of the American Anthropological Association, December 1, Washington, DC.

Islam, S. M., and Gronlund, A. G. 2011. Factors influencing the adoption of mobile phones among the farmers in Bangladesh: theories and practices. International Journal on Advances in ICT for Emerging Regions (ICTer) 4(1): 4-14.

Jackson, L.F. & Sullivan, C.V. 1995. Reproduction of white perch: the annual gametogenic cycle. Transactions of the American Fisheries Society 124(4):563-577.

Jochem, Eberhard. 2009. Improving the Efficiency of R&D and the Market Diffusion of Energy Technologies. New York, Springer Dordrecht. Kalusopa, T. 2005. The challenges of utilizing information communication technologies (ICTs) for the small-scale farmers in Zambia. Library Hi Tech 23(3): 414-424.

Katengeza, S. P., Juma, Okello, J., and Jambo, N. 2013. Use of mobile phone technology in agricultural marketing: The case of smallholder farmers in Malawi. International Journal of ICT Research and Development in Africa 2(2): 14-25.

Kirui, O.K., Okello, J.J, and Nyikal, R, A. 2012. Impact of mobile phone-based money transfer services in agriculture: evidence from Kenya. Selected paper presented to the International Association of Agricultural Economists (IAAE) triennial conference, Foz do Iguaçu, Brazil, 18-24 August, 2012.

Knowler, D. 2002. A review of selected bioeconomic models with environmental influences in fisheries. Journal of Bioeconomics 4:163-181.

Kwaku, K., P. A., Kweku, P. A., and Le Maire, P. 2006. Transforming recent gains in the digital divide into digital opportunities: Africa and the boom in mobile phone.

Kyomuhenedo P. 2002. A bioeconomic model for Uganda's Lake Victoria Nile Perch fishery. Master of Science in International Fisheries Management Thesis Universitetet-i-Tromso, Norway Litoselliti, L. 2003. Using Focused Groups in Research. London: Bloomsbury Academic.

Mai, K.,Yu, H., Ma, H., Duan, Q., Gisbert, E., Zamboni no Infante, J.L. & Cahu, C. 2005. A histological study of the development of the digestive system of Pseudosciaena crocea larvae and juveniles. Journal of Fish Biology, 67 (4): 1094-1106.

Manyala, J. O., K. Fitzsimmons, C. Ngugi, J. Ani, E. Obado. 2015. FIR on the Development of Aquaponics in Kenya. USAID Prime Award No. RDo11G-G Sub-Contract 1--AGR- 360844-UE

Maranya M.M., Luboobi L.S. Kuznetsosn D. 2014. Bioeconomic model for tilapia –Nile perch in polluted environment with constant harvesting efforts in Tanzanian waters of Lake Victoria. Mathematical Theory and Modeling 4 (7):28-42.

Martin, B and Abbott, E. 2010. Development calling: the use of mobile phones in agriculture development in Uganda. Mimeo. Greenlee School of Journalism and Communication. Ames: Iowa State University. Retrieved August 20, 2014, from: http://ifap-isobservatory.ittk.hu/trackback/442.

Masser M.P. 1997. Cage culture, site selection and water quality. SRAC Publications no 161.

Masuki, K.F.G., Kamugisha, R., Mowo, J.G., Tanui, J., Tukahirwa, J., Mogoi, J. and Adera, E.O. 2010. Role of mobile phones in improving communication and information delivery for agricultural development: lessons from South Western Uganda. Paper presented to Workshop at Makerere University, Uganda 22-23 March 2010. International Federation of Information Processing (IFIP), Technical Commission 9. Retrieved April 21, 2015, from: http://mak.ac.ug/documents/IFIP/RoleofMobilePhonesAgriculture.pdf.

Matuha, Moureen. 2015. Mobile phone use in Ugandan aquaculture: farmer experiences and aspirations. M.S. Thesis. Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences. Auburn, Alabama, USA.

Mclemore Ragan. 2011. Bioeconomic factors affecting feasibility of floating in pond-raceway, systems a Stella modeling approach. MSc Auburn University, Alabama.

Molony, T. 2008. Running out of credit: the limitations of mobile telephony in a Tanzanian agricultural marketing system. Journal of Modern African Studies. 46(4): 637–658.

Munguti, J. M., Charo-Karisa, H., Opiyo, M. A., M. Ogello, E. O., Marijani, E., Nzayisenga, L. 2012. Nutritive value and availability of commonly used feed ingredients for farmed Nile Tilapia (Oreochromis niloticus L.) and African catfish (Clarias gariepinus, burchell) in Kenya, Rwanda and Tanzania. African Journal of Food Agriculture, Nutrition and Development, 12(3): 1 – 22.

Muto, M and Yamano, T. 2009. The impact of mobile phone coverage expansion on market participation: Panel data evidence from Uganda. World Development 37(12):1887- 1896.

Myhr, J., and Nordstrom, P. 2008. Livelihood changes enabled by mobile phones: the case of Tanzania Fishermen. Uppsala University: Department of Business Studies. Retrieved February 06, 2015, from:www.divaportal.org/smash/get/diva2:131579/FULLTEXT01.pdf.

Natcher, W.C., & R. Weaver. 1999. The transmission of price volatility in the beef market: A multivariate approach. Paper selected for presentation at the American Agricultural Economics Association annual meeting, Nashville, TN.

NRC. 1993. Nutrient Requirement of Fish. National Research Council, Committee on Animal Nutrition, Board of Agriculture. Washington D.C., National Academic Press.

NRC. 2011. Nutrient requirements of fish and shrimp. Animal Nutrition Series, National Research Council of the National Academies. The National Academies Press, Washington, D.C., USA.376 pg.

Nunes, A, Marcelo V.C. Browdy. L., Vazquez-Anon, M. 2014. Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture 431 pg. 20–27

Oketch, M. L. 2015. Uganda: mobile money accounts increase by 2.1 million. The Monitor 16 December. Accessed 1-16-16: http://allafrica.com/stories/201512161162.html Open Signal. 2016.

Profile of Uganda cell phone network speeds and coverage. Accessed 1/17/2016. http://opensignal.com/networks/uganda/mtn-coverage?output=uganda/mtnuganda-coverage.

Overa, R. 2006. Networks, distance, and trust: telecommunications development and changing trading practices in Ghana. World Development 34 (7): 1301-1315. Retrieved March 10, 2015, from: http://dx.doi.org/10.1016/j.worlddev.2005.11.015.

Patino, R. & Sullivan, C.V. 2002. Ovarian follicle growth, maturation, and ovulation in teleost fish. Fish Physiology and Biochemistry 26(1):57-70. Perman, R, Y Ma, J., McGilvray & M. 1999. Common, Natural Resource And Environmental Economics, 2nd edition. Pearson Education Limited, Harlow

Reading, B.J., Hiramatsu, N., Sawaguchi, S., Matsubara, T., Hara, A., Lively, M.O., & Sullivan, C.V. 2009. Conserved and variant molecular and functional features of multiple egg yolk precursor proteins (vitellogenins) in white perch (Morone americana) and other teleosts. Marine Biotechnology 11(2):169-187.

Reading, B.J. and Sullivan, C.V. 2011. Chapter 257: Vitellogenesis in Fishes. In Encyclopedia of Fish Physiology: From Genome to Environment. A.P. Ferrell (Ed.). Elsevier, Maryland Heights, Missouri. 2272 pp.

Reading, B.J. & Sullivan, C.V. 2011. Chapter 257: Vitellogenesis in Fishes. In Encyclopedia of Fish Physiology: From Genome to Environment. A.P. Ferrell (Ed.). Elsevier, Maryland Heights, Missouri. 2272 pp.

Reading, B.J., Chapman, R.W., Schaff, J.E., Scholl, E.H., Opperman, C.H., and Sullivan, C.V. 2012. An ovary transcriptome for all maturational stages of the striped bass (Morone saxatilis), a highly advanced perciform fish. BMC Research Notes 5:111.

Reading, B.J., Williams, V.N., Chapman, R.W., Islam Williams, T., and Sullivan, C.V. 2013. Dynamics of the striped bass (Morone saxatilis) ovary proteome reveal a complex network of the translasome. Journal of Proteome Research 12:1691-1699.

Reading, B.J., Hiramatsu, N., Schilling, J., Molloy, K.T., Glassbrook, N., Mizuta, H., Luo, W., Baltzegar, D.A., Williams, V.N., Hara, A., and Sullivan, C.V. 2014. Lrp13 is a novel vertebrate lipoprotein receptor that binds vitellogenins in teleost fishes. Journal of Lipid Research 55(11):2287-2295. Undergraduate author.

Reading, B.J. and Schilling, J. (with Sullivan, C.V.). In press. Vitellogenesis in Fishes. In Reference Module in Life Sciences 2017 Edition. Elsevier, Maryland Heights, Missouri.

Rutaisire, J., Charo-karisa, H., Shoko, A. P., Nyandat, B., 2009. Aquaculture for increased fish production in East Africa. African Journal of Tropical Hydrobiology and Fisheries, 12(October): 74-77.

Salger, S.A., Cassady, K.R., Reading, B.J., and Noga, E.J. Accepted pending minor revision. A diverse family of host-defense peptides (piscidins) exhibit specialized anti-bacterial and anti-protozoal activities in fishes. PLoS ONE. Undergraduate author.

Sarnissa. 2010. Sustainable aquaculture research networks in sub-Saharan Africa: Synthesis report: assessment of national aquaculture programmes and policies in sub-Saharan Africa.

Stirling UK: Stirling University. Retrieved April 22, 2015, from: http://www.ruaf.org/sites/default/files/Synthesis%20report%20aquaculture%20policy%20reviews%20final_1.pdf.

Schilling, J., Loziuk, P.L., Muddiman, D.C., Daniels, H.V., & Reading, B.J. 2015. Mechanisms of Egg Yolk Formation and Implications on Early Life History of White Perch (Morone americana). PloS ONE 10(11):e0143225

Schilling, J., Loziuk, P.L., Muddiman, D.C., Daniels, H.V., and Reading, B.J. 2015. Mechanisms of Egg Yolk Formation and Implications on Early Life History of White Perch (Morone americana). PLoS ONE 10(11): e0143225. doi:10.1371/journal.pone.0143225

Serra, T. 2011. Food scares and price volatility: the case of the BSE in Spain. Food policy, 36(2): 179–185. Smith Homer W 1931. Observations on the African Lung-Fish, Protopterus Aethiopicus, and on Evolution from Water to Land Environments. Ecology 12, (1): 164-181.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A. andLovatelli, A. 2014. Small-scale aquaponic food production. Integrated fish and plant farming. FAO Fisheries and Aquaculture Technical Paper No. 589. Rome, FAO. 262 pp

Ssebisubi Maurice 2015. Analysis of small-scale fisheries' value-chains in Uganda. Kampala Uganda: Aquaculture Management Consultants Ltd. Available at: http://www.fao.org/valuechaininsmallscalefisheries/participatingcountries/uganda/en/

Ssebisubi, M. 2011. Analysis of small-scale fisheries' value-chains in Uganda. Unpublished Paper. Retrieved January 3, 2015, from: http://www.fao.org/valuechaininsmallscalefisheries/participatingcountries/uganda/it/.

Sullivan C.V., Berlinsky, D.L., & Hodson, R.G. 1997. Chapter 2: Reproduction. In Striped Bass and Other Morone Culture. R.M. Harrell (Ed.). Elsevier, Maryland Heights, Missouri. 2272 pp.

Swanson, B. E, Bentz, R. P, and Sofranko, J. A. 1997. Improving agricultural extension. a reference manual. Food and Agriculture Organization of the United Nations, Rome, Italy.

Tacon, A.G.J., Metian, M., Hasan, M.R. 2009. Feed ingredients and fertilizers for aquatic animals: sources and composition. FAO Fisheries and Aquaculture Technical Paper. No. 540. 209 pp. Teletchea, F. & Fontaine, P. 2014. Levels of domestication in fish: implications for the sustainable future of aquaculture. Fish and Fisheries 15(2):181-195.

Tacon, A.G.J., Hasan, M.R., Metian, M. 2011. Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects. FAO Fisheries and Aquaculture Technical Paper No. 564. 87 pp.

Timmers, B. 2012. Impacts of Climate Change and Variability on Fish Value Chains in Uganda. Project Report 2012-18. Penanag, Malaysia: World Fish Center. Available at: http://www.worldfishcenter.org/content/impacts-climate-change-and-variability-fishvalue-chains-uganda

Timuray, S. 2014. Mobile phones to boost productivity and incomes of 30,000 Tanzanian farmers. African farming and food processing magazine. London: Alain Charles Publishers Ltd. p.31. Retrieved June 18, 2015, from:http://www.africanfarming.net/crops/agriculture/mobile-phones-to-boostproductivityand-incomes-of-30-000-tanzanian-farmers.

Vijayakumar, C., Sridhar, S. and Haniffa, M.A. 1998. Low cost breeding and hatching techniques of the catfish (Heteropneustes fossilis) for small-scale farmers. Naga 21: 15-17.

Wallace, R.A. & Selman, K. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist 21:325-343. Whitten S. and Bennet J. 2000. Towards a bioeconomic model of wetland protection on private lands. Paper Presented at the 44th Annual Conference of the Australian Agricultural and Resource Economic Society.

Williams, V.N., Reading, B.J., Amano, H., Hiramatsu, N., Schilling, J., Islam Williams, T., Gross, K., and Sullivan, C.V. 2014. Proportional accumulation of yolk proteins derived from multiple vitellogenins is precisely regulated during vitellogenesis in striped bass (Morone saxatilis). Journal of Experimental Zoology Part A 321(6):301-315. (Research Article Highlighted on Journal Cover).

World Bank. 2013. Module 3: mobile devices and their impact. World Bank: ICT in agriculture source book. Retrieved August 20, 2014, from: http://www.ictinagriculture.org/sourcebook/module-3-mobile-devices-and-their-impact

World Bank. 2013. Using ICT to improve agricultural technology and market information dissemination to farmers in Indonesia. South-South Knowledge Exchange Hub. Retrieved May 13, 2015, from: http://wbi.worldbank.org/sske/story/using-ict-improveagricultural-technology-and-market-information-dissemination-farmers.

Yeo, I. K., & Lim, Y. K. 2015. Enzyme-linked immunosorbent assays (ELISA) and immunochromatography assays (ICG) for analysis of vitellogenin in the scorpion fish sebastiscus marmoratus. Korean Journal of Fisheries and Aquatic Sciences, 48(4): 459-465.

Yoo, K. H. and C. E. Boyd. 1994. Hydrology and water supply for pond aquaculture. Chapman and Hall, New York, New York.

Appendix II: Acronyms

Program-Related

ACRSP	Pond Dynamics/Aquaculture CRSP
AFCRSP	Aquaculture and Fisheries CRSP
AquaFish	Aquaculture and Fisheries CRSP
CRSP	Collaborative Research Support Program
HC	Host Country
ME	Management Entity
MO	Management Office
MOU	Memorandum of Understanding
NGO	Nongovernmental organization
PD/ACRSP	Pond Dynamics/Aquaculture CRSP
PI	Principal Investigator
RFA	Request for Assistance
RFP	Request for Proposals

General

FAQ	Frequently Asked Questions
KSh	Kenya Shillings
NB	Nota Bene, note well
PDF	Portable Document Format

Institutions, Organizations, Government Entities and Programs

AFU	Agriculture and Forestry University, Nepal
AOAC	Association of Analytical Chemists
APHA	American Public Health Association
ASEAN	Association of Southeast Asian Nations
BAU	Bangladesh Aquacultural University
BCSC	Bioinformatics Consulting and Service Core (NCSU)
CARP	Center for Aquaculture Research and Production, Nepal
CRC/URI	Coastal Resources Center/University of Rhode Island
CTU	Can Tho University, Vietnam
DASP	Department of Animal Sciences and Production, SUA
DoFD	Directorate of Fisheries Development, Nepal
EGAT	Bureau for Economic Growth, Agriculture, and Trade (USAID)
EPA	US Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization, United Nations
FARDeC	Fisheries and Aquaculture Research and Development Center, Cambodia
FiA	Fisheries Administration, Cambodia
FTF	Feed the Future (USG)
FTFMS	Feed the Future Monitoring System (USG)
FTI	Fisheries Training Institute, Uganda
GSL	Genomic Science Laboratory (NCSU)
IAAS	Institute of Agriculture and Animal Science, Nepal
IARC	International Agricultural Research Center(s), CGIAR
ICLARM	International Center for Living Aquatic Resources Management (The WorldFish Center), Malaysia
IFPRI	International Food Policy Research Institute

IFREDI Inland Fisheries Research and Development Institute, Cambodia
ILCC Industrial Library Center, Cambodia
IRB Internal Review Board
ISTA International Symposium on Tilapia in Aquaculture
IUCN International Union for the Conservation of Nature
KMFRI Kenya Marine and Fisheries Research Institute
KNUST Kwame Nkrumah University of Science and Technology, Ghana
MFAD Ministry of Fisheries and Aquaculture Development, Ghana
MRC Mekong River Commission
MSU Michigan State University
NACA Network of Aquaculture Centers in Asia, Thailand
NaFIRRI National Fisheries Resources Research Institute, Uganda
NARC Nepal Agricultural Research Council
NCSU North Carolina State University
NOAA National Oceanographic and Atmospheric Administration, USA
NRC National Research Council
NSF National Science Foundation, USA
OSU Oregon State University
PACRC Pacific Aquaculture and Coastal Resources Center/University of Hawai'i at Hilo
SRDI Soil Resources Development Institute (Ministry of Agriculture, Bangladesh)
SUA Sokoine University of Agriculture, Tanzania
SUCCESS Sustainable Coastal Communities and Ecosystems (EGAT/USAID)
UA University of Arizona
UAPB University of Arkansas, Pine Bluff
UHH University of Hawai'i at Hilo
UM The University of Michigan
UN United Nations
UNICEF United Nations International Children's Emergency Fund
URI University of Rhode Island
US United States
USA United States of America
USG United States Government
USAID United States Agency for International Development
USEPA US Environmental Protection Agency, USAVT Virginia Polytechnic Institute and State University
WFP World Food Program
WIOMSA Western Indian Ocean Marine Science Association

AquaFish Topic Areas

BMA Production System Design and Best Management Alternatives
FSV Food Safety, Post Harvest, and Value-Added Product Development
HHI Human Nutrition and Human Health Impacts of Aquaculture
IND Climate Change Adaptation: Indigenous Species Development
MER Marketing, Economic Risk Assessment and Trade
MNE Mitigating Negative Environmental Impacts
QSD Quality Seedstock Development
SFT Sustainable Feed Technology
PDV Policy Development
WIZ Watershed and Integrated Coastal Zone Management

Appendix II: Acronyms

<u>Scientific Terms</u>

AA	Ascorbic acid
ANOVA	Analysis of variance
CDHS	Cambodia Demographic Health Survey
CRD	Completely random design
DAP	Diammonium phosphate
DEG	Digitally expressed genes
DO	Dissolved oxygen
ELISA	Enzyme-linked immunosorbent assay
FBW	Final body weight
FCR	Feed conversion ratio
FCS	Food consumption score
FM	Fish meal
FMIS	Fish Market Information System
FYM	Farm yard manure
GDP	Gross domestic product
GLSS	Ghana Living Standards Score
GMO	Genetically modified organism
GNH	Groundnut husk
HACCP	Hazard Analysis and Critical Control Point
HPC	High performance computing
ICT	Information communication technologies
IPM	Integrated pest management
MT	Metric tons
NPP	Net primary productivity
PCR	Polymerase chain reaction
PER	Protein efficiency ratio
OAA	Other Aquatic Animals
OUT	Operational taxonomic unit
SDD	Sechi disk depth
SGR	Specific growth rate
SIS	Small indigenous fish species
SPC	Soy protein concentrate
SSF	Small sized fish
TDS	Total dissolved solids
THBS	Tanzania Household Budget Survey
TKN	Total Kjeldahl Nitrogen
TSS	Total suspended solids
VCA	Value Chain Analysis
WWG	Wet weight gain