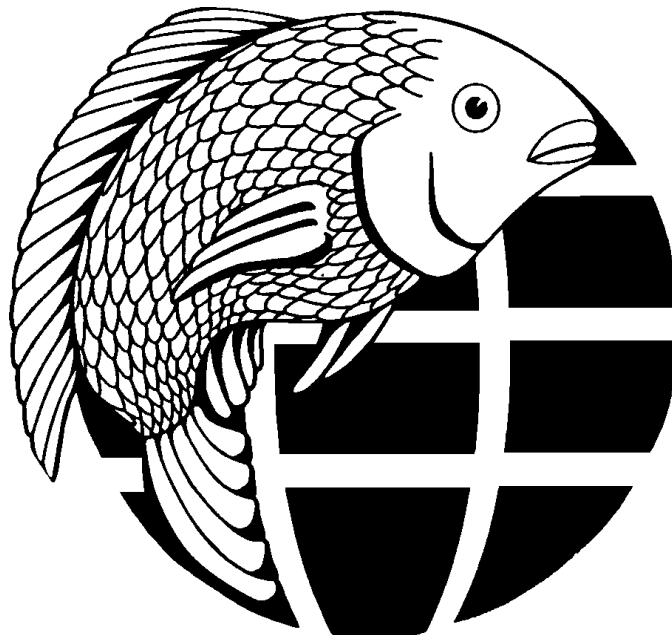


**Aquaculture  
Collaborative Research  
Support Program**

**ELEVENTH WORK PLAN**

1 May 2003 through 31 July 2004



Aquaculture CRSP  
Oregon State University  
418 Snell Hall  
Corvallis, OR 97331-1643

# **Aquaculture Collaborative Research Support Program**

## **ELEVENTH WORK PLAN**

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This work plan describes a standardized set of experiments to be undertaken by the Aquaculture Collaborative Research Support Program through July 2004, the end of the current grant period. Program activities are funded in part by Grant No. LAG-G-00-96-90015-00 from the United States Agency for International Development (USAID) and by participating US and host country institutions. The authors' opinions expressed herein do not necessarily reflect the views of USAID.

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The Eleventh Work Plan of the Aquaculture CRSP describes research activities to be conducted by the CRSP from 1 May 2003 through 31 July 2004 under United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00.

The goal of the current work period is to provide a basis for improving the sustainability of aquaculture production systems. The overall research context for the Eleventh Work Plan is aquaculture development in coastal and inland areas. Investigations fall into one of three program areas as they relate to sustainable aquaculture:

- Production Technology;
- Watershed Management; and
- Human Welfare, Health, and Nutrition.

All investigations describe aquaculture research that fosters economic growth, food security, and the wise use of natural resources. Themes addressed under the Eleventh Work Plan are described on P. 6. The table on pages 2 and 3 illustrates the distribution of Eleventh Work Plan investigations among program areas. The table on pages 4 and 5 illustrates the distribution of Eleventh Work Plan investigations among host countries.

### **Development of the Work Plan**

The CRSP's current grant was originally proposed as a five-year program, spanning from 1 August 1996 through 31 July 2001. After the award of a two-year extension, the grant completion date became 31 July 2003.

The CRSP disseminated the Eleventh Work Plan RFP on 1 August 2002. At that time, the CRSP was slated to submit a new five-year grant proposal to USAID that would initiate new activities as of 1 August 2003. One facet of developing the new proposal included identifying regional constraints to the success of small-holder fish farms. To that end the CRSP hosted a series of meetings. The first meeting was a stakeholder meeting held in Honduras. In the second phase of information gathering, the CRSP held three regional—Latin America and the Caribbean, Africa, and Asia—Expert Panel meetings. The constraints identified by these meetings formed the basis for the CRSP to develop a synthesized set of global researchable priorities for the 2003-2008 proposal.

The Eleventh Work Plan RFP called for two-year proposals that, once awarded, would have constituted the first two years of the new five-year grant. Proposals underwent an NSF-style review process. In the middle of the proposal review process, the CRSP learned that the program would receive a one-year extension of its current grant rather than consideration of a five-year proposal. Because of the foreshortened work plan duration, successful proponents to the Eleventh Work Plan RFP were required to revise their work plans and to resubmit one- rather than two-year plans.

RESEARCH AREAS AND THEMES REPRESENTED IN THE ELEVENTH WORK PLAN

Research Theme	Investigation Title	Research Code	Collaborators		Host Country/ies	Page
			US	Host Country		
<b>PRODUCTION TECHNOLOGY</b>						
Environmental Impacts Analysis	Co-Culture of Lotus and Hybrid Catfish to Recycle Wastes from Intensive Feeding	11EIAR1	Diana	Yi	Thailand	7
	Sustainable Development and Food Security	11SDFR1	C. Kohler, S. Kohler	Alcántara, del Aguilera, Rebaza, Tello	Bolivia, Brazil, Colombia, Ecuador, and Peru	13
	Tilapia ( <i>Oreochromis niloticus</i> ) Production Constraints in Bangladesh	11SDFR2	Diana, Lin	Yi, Bart, Shivakoti, Wahab	Bangladesh	18
	Reproductive Performance and Growth of Improved Tilapia, <i>Oreochromis niloticus</i>	11SDFR3	Diana	Bart, Mair	Thailand	20
Production System Design and Integration	New Paradigm in Farming of Freshwater Prawn ( <i>Macrobrachium rosenbergii</i> ) with Closed and Recycle Systems	11PSDR2	Diana, Lin	Yi	Thailand	29
	Integrated Cage-Cum-Pond Culture Systems with High-Valued Fish Species in Cages and Low-Valued Species in Open Ponds	11PSDR3	Diana, Lin	Yi, Wahab, Shrestha, Phuong	Thailand, Bangladesh, Nepal, Vietnam	32
	Mitigating Environmental Impact of Cage Culture through Integrated Cage-Cum-Cove Culture System in Tri An Reservoir of Vietnam	11PSDR4	Diana, Lin	Yi, Hung	Vietnam	35
	Optimization of Nitrogen Fertilization Regime in Fertilized Nile Tilapia Ponds With Supplemental Feed	11PSDR5	Diana, Lin	Yi	Thailand	38
Indigenous Species Development	Workshop on Fertilization Strategies for Pond Culture in Bangladesh	11PSDR6	Diana, Lin	Yi, Wahab	Bangladesh	41
	Broodstock Development and Larval Feeding of Amazonian Fishes	11ISDR1	C. Kohler, S. Kohler, Abiado, Dabrowski, Rincharad	Alcántara, Tello	Peru	44
Water Quality and Availability	Controlled Reproduction of an Important Indigenous Species, <i>Spiribarbus denticulatus</i> , in Southeast Asia	11ISDR2	Diana	Bart, Trung	Vietnam	48
	Pond Design and Watershed Analyses Training	11WQAR3	Tollner	Meyer, Pilz	Honduras, Panama	63
Economic/Risk Assessment and Social Analysis	Preliminary Work on Site Description, Evaluation, and Development Planning: Tanzania, Ghana, and Kenya	11ERAR1	Quagraaine, Duke	Gitonga, Amisah, Katule	Kenya, Ghana, Tanzania	71
Applied Technology and Extension Methodologies	Aquaculture CRSP Sponsorship of the Sixth International Symposium on Tilapia in Aquaculture	11ATER2	Fitzsimmons	Bolivar	Global	77
	Aquaculture CRSP—Global Contributions to Sustainable Aquaculture: A Special Session at the 2004 World Aquaculture Conference	11ATER3	Fitzsimmons		Global	79
	Aquaculture Training for Kenyan Fisheries Assistants	11ATER4	Bowman	Ngugi, Omolo	Kenya	81

**RESEARCH AREAS AND THEMES REPRESENTED IN THE ELEVENTH WORK PLAN (CONTINUED)**

Research Theme	Investigation Title	Research Code	Collaborators		Host Country/ies	Page
			US	Host Country		
<b>PRODUCTION TECHNOLOGY (CONTINUED)</b>						
Seedstock Development and Availability	Mitigating the Effects of High Temperature and Turbidity on Seed Production of Nile Tilapia from Hapa-in-Pond Systems	11SSDR1	Diana, Rakocy	Yi	Thailand	84
	Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras	11SSDR2	Molnar	Meyer	Honduras, Guatemala, El Salvador, Nicaragua	88
Fish Nutrition and Feed Technology	Nutrition and Nutrient Utilization in Native Peruvian Fishes	11FNFR1	C. Kohler, Lochmann, S. Kohler	Alcántara, Padilla, Tello	Peru	94
	Evaluation of Tilapia Aquaculture Best Practices in Central Luzon, the Philippin	11FNFR2	Brown	Bolivar	Philippines	98
	Use of Phytochemicals as an Environmentally-Friendly Method to Sex-Reverse Nile Tilapia	11FNFR3	Dabrowski	Contreras, Márquez, Páramo	Mexico	101
	Insulin-like Growth Factor-I as a Growth Indicator in Tilapia	11FNFR4	Brown, Borski	Bolivar	Philippines	107
<b>WATERSHED MANAGEMENT</b>						
Environmental Impacts Analysis	Further Studies on Soil Quality in Aquaculture Ponds in Thailand	11EIAR2	Boyd	Boonyaratpalin	Thailand	10
Production System Design and Integration	Diversification Into Sustainable Tilapia-Shrimp Polyculture and Small-Scale Tilapia Cage Culture in Mexico	11PSDR1	Fitzsimmons	Duncan	Mexico	24
Indigenous Species Development	Development of Aquaculture Techniques for the Indigenous Species of Southern Mexico, <i>Centropomus undecimalis</i> : Sex Determination and Differentiation and Effects of Temperature	11ISDR3	Patino, Pope	Contreras, Márquez, Páramo	Mexico	51
Water Quality and Availability	Elimination of Methyltestosterone from Intensive Masculinization Systems: Use of Ultraviolet Irradiation of Water	11WQAR1	Schreck, Giannico	Contreras, Márquez	Mexico	55
	Use of a Wetland to Treat Effluent from a Flow-Through Aquaculture System	11WQAR2	Boyd	Sipatuba-Tavares	Brazil	60
	Influence of Daily Feed Allowance on Pond Water and Effluent Quality	11WQAR4	Boyd, Lim	Salie, de Wet	South Africa	67
Applied Technology and Extension Methodologies	Training Local Farmers on Safe Handling of Steroids and Masculinization Techniques in Central America	11ATER1	Schreck, Giannico	Contreras, Perez	Mexico	74
Seedstock Development and Availability	Continuation of a Selective Breeding Program for Nile Tilapia to Provide Quality Broodstock for Central America	11SSDR3	Schreck, Giannico	Contreras, Fernández	Mexico	91

**HOST COUNTRIES PARTICIPATING IN ELEVENTH WORK PLAN INVESTIGATIONS**

Host Country	Investigation Title	Research Code	Collaborators		Page
			US	Host Country	
<b>MEXICO</b>	Diversification Into Sustainable Tilapia-Shrimp Polyculture and Small-Scale Tilapia Cage Culture in Mexico	11PSDR1	Fitzsimmons	Duncan	24
	Development of Aquaculture Techniques for the Indigenous Species of Southern Mexico, <i>Centropomus undecimalis</i> : Sex Determination and Differentiation and Effects of Temperature	11ISDR3	Patino, Pope	Contreras, Márquez, Páramo	51
	Elimination of Methyltestosterone from Intensive Masculinization Systems: Use of Activated Charcoal in Concrete Tanks	11WQAR1	Schreck, Giannico	Contreras, Márquez	55
	Training Local Farmers on Safe Handling of Steroids and Masculinization Techniques in Central America	11ATER1	Schreck, Giannico	Contreras, Perez	74
	Continuation of a Selective Breeding Program for Nile Tilapia to Provide Quality Broodstock for Central America	11SSDR3	Schreck, Giannico	Contreras, Fernández	91
<b>HONDURAS</b>	Use of Phytochemicals as an Environmentally-Friendly Method to Sex-Reverse Nile Tilapia	11FNFR3	Dabrowski	Contreras, Márquez, Páramo	101
	Pond Design and Watershed Analyses Training	11WQAR3	Tollner	Meyer, Pilz	63
	Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras	11SSDR2	Molnar	Meyer	88
<b>BOLIVIA</b>	Amazon Aquaculture Outreach	11SDFR1	C. Kohler, S. Kohler	Alcántara, Rebaza, del Aguila, Tello	13
<b>BRAZIL</b>	Amazon Aquaculture Outreach	11SDFR1	C. Kohler, S. Kohler	Alcántara, Rebaza, del Aguila, Tello	13
<b>EL SALVADOR</b>	Use of a Wetland to Treat Effluent from a Flow-Through Aquaculture System	11WQAR2	Boyd	Sipaúba-Tavares	60
	Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras	11SSDR2	Molnar	Meyer	88
<b>GUATEMALA</b>	Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras	11SSDR2	Molnar	Meyer	88
<b>NICARAGUA</b>	Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras	11SSDR2	Molnar	Meyer	88
<b>PANAMA</b>	Pond Design and Watershed Analyses Training	11WQAR3	Tollner	Meyer, Pilz	63
<b>PERU</b>	Amazon Aquaculture Outreach	11SDFR1	C. Kohler, S. Kohler	Alcántara, Rebaza, del Aguila, Tello	13
	Broodstock Development and Larval Feeding of Amazonian Fishes	11ISDR1	C. Kohler, S. Kohler, Dabrowski, Abiado, Rincharid	Tello, Alcántara	44
<b>COLOMBIA</b>	Nutrition and Nutrient Utilization in Native Peruvian Fishes	11FNFR1	C. Kohler, S. Kohler, Lochmann	Tello, Alcántara, Padilla	94
	Amazon Aquaculture Outreach	11SDFR1	C. Kohler, S. Kohler	Alcántara, Rebaza, del Aguila, Tello	13
<b>ECUADOR</b>	Amazon Aquaculture Outreach	11SDFR1	C. Kohler, S. Kohler	Alcántara, Rebaza, del Aguila, Tello	13



## HOST COUNTRIES PARTICIPATING IN ELEVENTH WORK PLAN INVESTIGATIONS (CONTINUED)

Host Country	Investigation Title	Research Code	Collaborators		Page
			US	Host Country	
<b>KENYA</b>	Preliminary Work on Site Description, Evaluation, and Development Planning: Tanzania, Ghana, and Kenya	11ERAR1	Quagraine, Duke	Gitonga, Ngugi	7
	Aquaculture Training for Kenyan Fisheries Assistants	11ATER4	Langdon, Bowman	Omolo, Ngugi	81
<b>GHANA</b>	Preliminary Work on Site Description, Evaluation, and Development Planning: Tanzania, Ghana, and Kenya	11ERAR1	Quagraine, Duke	Amisah, Kumah	7
<b>TANZANIA</b>	Preliminary Work on Site Description, Evaluation, and Development Planning: Tanzania, Ghana, and Kenya	11ERAR1	Quagraine, Duke	Katule, Ngatunga, Mapunda	7
<b>SOUTH AFRICA</b>	Influence of Daily Feed Allowance on Pond Water and Effluent Quality	11WQAR4	Boyd, Lim	Salie, de Wet	67
<b>BANGLADESH</b>	Tilapia ( <i>Oreochromis niloticus</i> ) Production Constraints in Bangladesh	11SDFR2	Diana, Lin	Yi, Bart, Shivakoti, Wahab	18
	Integrated Cage-Cum-Pond Culture Systems with High-Valued Fish Species in Cages and Low-Valued Species in Open Ponds	11PSDR3	Diana, Lin	Wahab	32
	Workshop on Fertilization Strategies for Pond Culture in Bangladesh	11PSDR6	Diana, Lin	Wahab, Yi	41
<b>NEPAL</b>	Integrated Cage-Cum-Pond Culture Systems with High-Valued Fish Species in Cages and Low-Valued Species in Open Ponds	11PSDR3	Diana, Lin	Shrestha	32
<b>PHILIPPINES</b>	Evaluation of Tilapia Aquaculture Best Practices in Central Luzon, the Philippines	11FNFR2	Brown	Bolivar	98
	Insulin-like Growth Factor-I as a Growth Indicator in Tilapia	11FNFR4	Brown, Borski	Bolivar	107
<b>THAILAND</b>	Co-Culture of Lotus and Hybrid Catfish to Recycle Wastes from Intensive Feeding	11EIAR1	Diana	Yi	7
	Further Studies on Soil Quality in Aquaculture Ponds in Thailand	11EIAR2	Boyd	Boonyaratpalin	10
	Reproductive Performance and Growth of Improved Tilapia, <i>Oreochromis niloticus</i>	11SDFR3	Diana	Bart, Mair	20
	New Paradigm in Farming of Freshwater Prawn ( <i>Macrobrachium rosenbergii</i> ) with Closed and Recycle Systems	11PSDR2	Diana	Lin, Yi	29
	Integrated Cage-Cum-Pond Culture Systems with High-Valued Fish Species in Cages and Low-Valued Species in Open Ponds	11PSDR3	Diana, Lin	Yi	32
	Optimization of Nitrogen Fertilization Regime in Fertilized Nile Tilapia Ponds With Supplemental Feed	11PSDR5	Diana, Lin	Yi	38
	Mitigating the Effects of High Temperature and Turbidity on Seed Production of Nile Tilapia from Hapa-in-Pond Systems	11SSDR1	Diana, Rakocy	Yi	84
<b>VIETNAM</b>	Integrated Cage-Cum-Pond Culture Systems with High-Valued Fish Species in Cages and Low-Valued Species in Open Ponds	11PSDR3	Diana, Lin	Phuong	32
	Mitigating Environmental Impact of Cage Culture through Integrated Cage-Cum-Cove Culture System in Tri An Reservoir of Vietnam	11PSDR4	Diana, Lin	Yi, Hung	35
	Controlled Reproduction of an Important Indigenous Species, <i>Spirinbarbus denticulatus</i> , in Southeast Asia	11ISDR2	Diana	Bart, Trung	48
<b>GLOBAL</b>	Aquaculture CRSP Sponsorship of the Sixth International Symposium on Tilapia in Aquaculture	11ATR2	Fitzsimmons	Bolivar	77
	Aquaculture CRSP—Global Contributions to Sustainable Aquaculture: A Special Session at the 2004 World Aquaculture Conference	11ATR3	Fitzsimmons		79

# ELEVENTH WORK PLAN RESEARCH THEMES

### **Environmental Impacts Analysis**

With the rapid growth in aquaculture production, environmental externalities are of increasing concern. Determining the scope and mitigating or eliminating the negative environmental impacts of aquaculture—such as poor management practices and the effects of industrial aquaculture—is a primary research goal of this program.

### **Sustainable Development and Food Security**

Aquaculture is increasing in importance as a source for poverty alleviation and food security in developing regions of the world. A focal area of the program is to support research related to sustainable aquatic farming systems that can demonstrably ensure a reliable future food supply.

### **Production System Design and Integration**

Aquaculture is an agricultural sector with specific input demands. Systems should be designed to improve efficiency and/or integrate aquaculture inputs and outputs with other agricultural and non-agricultural production systems.

### **Indigenous Species Development**

Domestication of new and indigenous species may contribute positively to the development of local communities as well as protect ecosystems. At the same time, the development of new species for aquaculture must be approached in a responsible manner that diminishes the chance for negative environmental, technical, and social impacts. Research that investigates relevant policies and practices is encouraged while exotic species development is not encouraged.

### **Water Quality and Availability**

Aquaculture development that makes wise use of natural resources is at the core of the CRSP. Research that yields better understandings of water and aquaculture is a matter of great interest. The range of research possibilities is broad—from investigations that quantify such things as availability and quality to those that look into the social context of water and aquaculture, including water rights, national and regional policies (or the lack of them), traditional versus industrial uses, and the like.

### **Economic/Risk Assessment and Social Analysis**

Aquaculture is a rapidly growing industry; its risks and impacts on society 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; and the availability of financial resources for small farmers.

### **Applied Technology and Extension Methodologies**

Developing appropriate technology and providing technology-related information to end-users is a high priority. The program encourages research that results in a better understanding of factors and practices that set the stage for near-term technology implementation and that contribute to the development of successful extension tools and methods.

### **Seedstock Development and Availability**

Procuring reliable supplies of high quality seed for stocking local and remote sites is critical to continued development of the industry. A better understanding of the factors that can contribute to stable seedstock quality and quantity for aquaculture enterprises is essential.

### **Disease, Predation Prevention, and Food Safety**

Protecting aquatic animals from diseases and predators and ensuring high quality, safe, and nutritious aquaculture products for local consumers and the competitive international marketplace is a primary research goal. Consumers and producers alike will benefit from research that contributes to the development of standards and practices that protect aquaculture products from spoilage, adulteration, mishandling, and off-flavors.

### **Fish Nutrition and Feed Technology**

Ways and methods of increasing the range of available ingredients and improving the technology available to manufacture and deliver feeds is an important research theme. Better information about fish nutrition can lead to the development of less expensive and more efficient feeds. Research that investigates successful adoption and extension strategies for the nutritional needs of fish is also encouraged.

### **Aquaculture and Human Health Impacts**

Aquaculture can be a crucial source of proteins and micronutrients for improved human health, growth, and development. Conversely, human health can be negatively impacted by aquaculture if it serves as a direct or indirect vector for human diseases. There is also interest in better understanding the interconnectedness of such human health crises as AIDS/HIV and aquaculture production. All of the above issues are research priorities.

## Co-Culture of Lotus and Hybrid Catfish to Recycle Wastes from Intensive Feeding

Environmental Impacts Analysis 1 (11EIAR1)/Experiment/Thailand

### Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology, Pathumthani, Thailand
James S. Diana	US Principal Investigator	The University of Michigan

### Objectives

- 1) Assess the nutrient recovery from feeding wastes by lotus plants.
- 2) Assess pond mud characteristics after lotus-fish culture.
- 3) Compare fish growth between monoculture and lotus-fish co-culture.

### Significance

Semi-intensive and intensive fish culture systems are characterized by applications of organic and inorganic fertilizers, supplemental by-product feeds, and high protein diets to achieve high production. After a period of continuous input of manures, inorganic fertilizers, and fish feed, organic matters and nutrients accumulate at high concentrations in pond mud. Green and Boyd (1995) reported that the nutrients accumulated in pond mud accounted for about 70% of total nitrogen (TN) and 35 to 40% of total phosphorus (TP) in intensively manured tilapia ponds. One hectare of old pond mud was reported to have the equivalent of 3.0 tons of triple superphosphate (TSP) and 2.8 tons of urea (Yang and Hu, 1989), 2.30 tons of TSP and 1.85 tons of urea (Shrestha and Lin, 1997) or 4.11 to 4.81 tons of TSP and 3.44 to 3.92 tons of urea (Yi et al., 2002) in fertilized ponds, and 12.4 to 12.9 tons of urea and 7.7 to 9.2 tons of TSP in intensively fed ponds (Mon, 2000). Although the absorbed nutrients, especially phosphorus (P), may release back to the water column, the amount is limited and most nutrients remain in the pond mud (Shrestha and Lin, 1996). Thus, recovering nutrients from pond mud is a major concern of waste recycling in aquaculture.

Pond mud has been widely used as fertilizer for land crops (Muller, 1978; Little and Muir, 1987; Christensen, 1989; Shrestha and Lin, 1997) due to their ability to absorb nutrients directly from mud (Denny, 1972; Boyd, 1982; Smart and Barko, 1985). However, removing pond mud is labor-intensive and its practicability is questionable (Edwards et al., 1986). Alternatively, rooted aquatic macrophytes may have great potential to be used to recycle adsorbed nutrients in pond mud. Shrestha and Lin (1997) used a rooted semi-aquatic plant, taro (*Colocasia esculenta*), to recover P contained in pond mud and concluded that P in pond mud was sufficient to support taro growth. In PD/A CRSP sponsored projects, Mon (2000) and Yi et al. (2002) also reported that nutrients contained in old pond mud were sufficient for the growth of lotus (*Nelumbo nucifera*).

Lotus is an emergent aquatic macrophyte and is commonly planted in nutrient-rich fields or ponds. Lotus is an important and popular cash crop in many Asian countries. Various parts of lotus plants can be used as foods, medicine, religious ornaments, fuel, and cosmetic ingredients. Lotus has been co-cultured or cultured rotationally with fish in China for many years (Hoffmann, 1934; cited by Edwards, 1987), and also rotated with tilapia seed production in some parts of the sewage-fed areas around Ho Chi Minh City, Vietnam (Little and Tuan, 1995). The rotation of fish and aquatic macrophytes may give farmers two crops to market rather than one (Edwards, 1987). However, little research has been done to determine the efficiency of nutrient removal from pond mud by lotus or its economic return. Recently, Yi et al. (2002) reported that the growth and survival of Nile tilapia (*Oreochromis niloticus*) was much lower in a lotus-tilapia co-culture system than that in tilapia monoculture due to lower phytoplankton biomass and poor water quality caused by shading effects of lotus leaves. They suggest that airbreathing species that can tolerate very low dissolved oxygen (DO) concentrations and are commonly cultured in intensive systems, may be the better choice for a lotus-fish co-culture system.

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The purposes of this study were to assess the feasibility for co-culture of lotus and hybrid catfish in intensively fed ponds, to assess the nutrient recovery from feed wastes by lotus plants, to assess pond mud characteristics after lotus-fish co-culture, and to compare fish growth in monoculture and in lotus-fish co-culture.

### Anticipated Benefits

Results of the experiment will provide information on a lotus-fish co-culture system and on recycling of nutrients in feeding wastes. It will generate information on bottom mud characteristics altered by rooted plants. It may benefit small-scale farmers of Asian countries for resource utilization where lotus is commonly grown as a cash crop.

### Research Design

*Location:* AIT.

*Methods:* Pond research.

*Pond Facility:* Nine earthen ponds of 200 m<sup>2</sup> size.

*Culture Period:* 180 days.

*Test Species:* Hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) and lotus (*Nelumbo nucifera*).

*Stocking Density:* Hybrid catfish at 25 fish per m<sup>2</sup>, lotus at one seedling per m<sup>2</sup>.

*Nutrient Input:* Pelleted feed (farmers' practice).

*Water Management:* After lotus planting, water level will be increased as the height of lotus plant increases. Once the water level reaches 30 cm, hybrid catfish fingerlings will be stocked. Water level will be increased with growth of lotus up to 1 m depth.

### *Sampling Schedule:*

Water quality: standard CRSP protocol, biweekly water quality sampling, and diel analysis at various depths.

Initial and final pond mud sampling for organic C, TN, and TP.

Hybrid catfish will be sampled at stocking and harvest, and pelleted feed will be sampled for every batch and analyzed for TN and TP.

Fish growth and survival will only be assessed at the end of the experiment due to sampling difficulties. Fish and lotus will be harvested by draining.

Nutrient budgets will be estimated for all ponds.

Partial enterprise budgets will be estimated for cost of inputs and value of fish and lotus.

### *Statistical Design, Null Hypothesis, and Statistical Analysis:*

Experiment will have three treatments in triplicates:

- 1) Lotus-hybrid catfish co-culture.
- 2) Hybrid catfish alone.
- 3) Lotus alone.

The null hypothesis is that there will be no differences for nutrient concentrations in pond mud, soil characteristics, fish growth, and nutrient recovery between two treatments.

The data will be analyzed using ANOVA.

**Regional Integration**

Lotus is a popular cash crop in many Asian countries. Hybrid catfish is commonly cultured in the region. Small-scale farmers are resource limited, and lotus-hybrid catfish co-culture may utilize waste nutrient resources otherwise.

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## Further Studies on Soil Quality in Aquaculture Ponds in Thailand

Environmental Impacts Analysis 2 (11EIAR2)/Study/Thailand

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### Objectives

- 1) Determine the quality of bottom soil in ponds for production of catfish (*Clarias* spp.), carp (*Puntius* spp.), and freshwater shrimp (*Macrobrachium rosenbergii*).
- 2) Ascertain the practices currently used for bottom soil management in the three types of fish culture.
- 3) Develop recommendations for enhancing pond bottom soil management.

### Significance

Pond bottom soil quality influences water quality and fish production in earthen ponds (Banerjee, 1967). There is a widely held opinion that pond bottom soils accumulate large amounts of organic matter, decrease in pH, and become higher in phosphorus and nitrogen concentrations over time (Boyd, 1995). Thus, fish and shrimp farmers dry pond bottoms between crops, resort to heavy applications of liming materials, and often remove sediment from ponds as means of enhancing bottom soil quality (Boyd et al., 2002). Although these practices may be needed in some cases, several studies of pond bottom soils have revealed that bottom soil quality does not deteriorate as rapidly as many fish and shrimp farmers believe (Tucker, 1985; Munsiri et al., 1995; Munsiri et al., 1996; Tepe and Boyd, 2002). Thus, pond bottom soil treatments should be applied as needed rather than as a routine effort after each crop.

A recent study funded by the PD/A CRSP showed that tilapia ponds in Thailand had reasonably good soil quality even after 30 to 40 years in production. Tilapia farmers in Thailand often use more liming materials in ponds than necessary. Sediment removal is practiced frequently, but it has not caused demonstrable reduction in soil organic matter concentrations. There are several other kinds of pond aquaculture in Thailand, the most important being production of catfish (*Clarias* spp.), carp (*Puntius* spp.), and freshwater shrimp (*Macrobrachium rosenbergii*). Thus, studies of bottom soil quality in ponds that have been used for different lengths of time for culture of these species would be useful in ascertaining if bottom soil quality characteristics and changes are similar to those observed in tilapia culture.

### Anticipated Benefits

The overall benefit expected from this project will be to expand the previous study on bottom soil quality in tilapia ponds to other types of pond aquaculture in Thailand. The specific benefits expected are as follows:

- 1) The results of the study will reveal if soil quality is similar among different types of pond aquaculture.
- 2) The findings will reveal if farmers are using liming materials efficiently and if current pond management procedures are adequate to maintain good soil quality.
- 3) General recommendations on pond bottom soil management for major types of freshwater fish and shrimp culture will be developed from this effort.

### Research Design

*Pond Facilities:* Ponds for use in this work will be located on private fish farms and at fisheries research stations in central Thailand. Ponds usually are 2,000 to 5,000 m<sup>2</sup> in area with average depths of about 1 to 1.5 m. Ponds that have only been used for culture of catfish, carp, and freshwater shrimp



will be sought. Moreover, only ponds that have been in continuous production for a known number of years will be selected. We plan to select at least 15 ponds for each species for use in the study, and if possible, 20 to 25 ponds will be used.

*Research Plan and Methodology:* Ponds of different (but known) ages will be located by the Thailand Department of Fisheries and will be farmer ponds or ponds on research stations. Information on pond management history for each pond will be obtained from owners or managers. Core samples will be collected from five places near the bottom of each pond with a 5 cm diameter core tube. The cores will be inspected, and the depths of the S-horizon and the total sediment depth will be measured. Munsiri et al. (1995) describe how to identify the thickness of the S-horizon and total sediment depth by visual inspection. The core segment representing the S-horizon will be pushed out of the core tube, cut, and saved in a plastic container. All cores from a pond will be combined to provide a single, composite sample. These samples will be oven dried at 60°C. A separate set of cores from the S-horizon will be placed in soil moisture cans of known weight and dried at 105°C for determination of dry bulk density (Blake and Hartge, 1986). Dry samples will be transported to Auburn University for analytical work. The samples dried at 60°C will be pulverized with a mechanical soil crusher to pass through a 40-mesh screen and saved for chemical analyses and determination of the reactivity of the organic matter.

Soil pH will be determined by glass electrode in 1:1 mixtures (weight:volume) of dry soil and distilled water (Thunjai et al., 2001). Exchangeable acidity will be measured by the procedure of Pillai and Boyd (1985), and the lime requirements estimated based on bulk density, depth of S-horizon, and exchangeable acidity.

Total carbon will be determined by Leco carbon analyzer and organic carbon will be measured by the Walkley-Black sulfuric acid-potassium dichromate oxidation method (Nelson and Sommers, 1982).

Total nitrogen, total phosphorus, phosphorus adsorption capacity, and total sulfur will be measured by methods described by Munsiri et al. (1995).

*Statistical Analysis:* The nature of this study does not allow for replication of pond ages as treatments. The data will be analyzed primarily by regression analysis using age as the dependent variable and soil quality variables as independent variables. Soil quality will be compared among ponds with different culture species by analysis of variance.

### **Regional Integration**

The project will integrate well into the regional plan. The changes that occur in fish pond soils over time are not expected to be country specific or even region specific. These changes should occur in all ponds in similar climatic areas that are managed in a similar way. Thus, the pond management information should be useful within the region and even outside of the region.

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## Amazon Aquaculture Outreach

Sustainable Development and Food Security 1 (11SDFR1)/  
Activity, Study/Bolivia, Brazil, Colombia, Ecuador, and Peru

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### **Objectives**

- 1) Provide extension services to the community to promote sustainable aquaculture in the Amazonian region.
- 2) Conduct demonstration projects with local fish farmers to expose them to new species and/or techniques.
- 3) Provide short courses to governmental and NGO personnel to maintain and expand a network of aquaculture extensionists in Peru and neighboring Amazonian countries.
- 4) Develop an exchange program for researchers, extensionists, and students in the Amazon region.
- 5) Maintain and expand the specialized website developed in Work Plan 10 on Amazonian aquaculture to provide for information exchange and networking.

### **Significance**

Fish culture has been practiced for over three decades in the Peruvian Amazon and for over fifty years in Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, and Venezuela (the Amazon region). The countries comprising the Amazon region are linked by major river systems, particularly the drainages comprising the Amazon and Orinoco Rivers. The largest diversity of freshwater fishes in the world is contained within these drainages.

In the Peruvian Amazon, the total pond surface area along the Iquitos–Nauta Road has increased from 22 ha in 1991 to slightly above 200 ha in 2002 (unpublished data, Alcántara, 2002). These fish farmers received support from public and private entities. A sociological study determined that over 80% of the human population along the Iquitos-Nauta Road and in the Tamishiyacu and Mazan River areas have great interest in aquaculture practices, and over 80% of practicing farmers saw aquaculture as more feasible than other forms of land farming (Molnar et al., 1999).

It has been only in the past two years that fish farmers along the Iquitos-Nauta road and the Santa Helena and Huayococha indigenous communities in Tigre River (Maynas Province, Loreto Department, Peru) have been properly supported with aquaculture extension activities. The food security program (PROSEAL) originally created and directed by Terra Nuova (NGO) and IIAP, initiated the extension service for these communities in 1999 with the assistance of five extension agents (three biologists and two technicians). After Terra Nuova completed its prime goals in December 2001, CRSP and IIAP took over this important task through a transitional period in 2001 by rehiring up to two of the five extension agents and by enrolling a third extension agent in a Ph.D. program at SIUC. PROSEAL's goal was to promote the organization of fish farmers into self-sustainable associations in order to develop the aquaculture industry in a coordinated form, allowing for vital farmer interactions and common education among them. Results from research conducted at our host country facilities provided much of the information that PROSEAL extended to farmers. Thus, PROSEAL was a direct beneficiary from the CRSP program in Peru. As a result of the technical support and outreach efforts of the CRSP/IIAP team in Work Plan 9 and Work Plan 10, valuable information has been transferred to the Iquitos and Tigre River area fish farmers.

In Work Plan 9, an extension work committee comprised of local biologists and fish farmers was created, allowing us to formally integrate our extension activities for Work Plan 11 into the existing host country Prime Site program and to expand this experience to other countries of the vast Amazonian region. The committee ensures proper cooperation among all participating entities and helps avoid redundancy in the proposed work region. Dr. Alcántara (former PROSEAL Technical Director), with CRSP support, will continue to serve as the lead aquaculture extensionist. The PROSEAL project terminated in December 2001. Accordingly, the continuity of this important effort will be reliant on CRSP support.

### **Quantified Anticipated Benefits**

The development of sustainable aquaculture will benefit many sectors throughout the Amazon region. Rural farmers will benefit from the addition of an alternative form of agriculture. Aquaculture production requires considerably less land than that needed for cattle ranching. Moreover, ponds can

be used year after year, whereas rain forest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons. Such lands, once abandoned, usually can no longer support normal jungle growth. Both rural and urban poor will benefit by the addition of a steady supply of high quality protein in the marketplace. Aquaculture of *Colossoma*, *Piaractus*, and *Arapaima* should relieve some of the fishing pressure on these overharvested native species. The two former genera have been suggested to play a crucial ecological role in disseminating seeds from the flooded forest (Goulding, 1980; Araujo-Lima and Goulding, 1997; Chu, dissertation in progress). Accordingly, the aquaculture of *Colossoma* and *Piaractus* may be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Peruvian Amazon. Host country consumers and fish farmers, researchers, extensionists and planners, local and foreign Latin-American governmental organizations and/or NGOs and users of global CRSP-sponsored models and data will benefit from this activity. Development of a Latin American network of Amazonian species producers and researchers has begun to catalyze regional efforts to fortify the growing industry and to explore new aquaculture candidates to diversify production in this highly productive and species-rich region. Specifically, we will quantify the following:

- 1) Number of fish farmers receiving extension services.
- 2) Number of training participants (extension agents, students, and experts from neighboring countries).
- 3) Number of "Master Aquaculturists" certified.
- 4) Number of on-farm research studies and demonstrations.
- 5) Number of hits at Amazonian aquaculture web page.

## Activity Plan

*Location of work:* This work will be conducted in Bolivia, Brazil, Colombia, Ecuador, and Peru.

## Methods

Objective 1: Provide extension services to the local community to promote sustainable Aquaculture in the Amazon region.

We will continue to reinforce extension activities with the local farmers currently being served along the road system between the cities of Iquitos and Nauta. We will expand further the coverage by moving an extension agent to Pucallpa. This will facilitate expanding coverage to other Peruvian localities as well as to initiate outreach activities through extensionists' exchange and training programs in other countries of the vast Amazonian region (see list of collaborators above). Farmers will be provided with knowledge gleaned from the CRSP-sponsored studies with *Colossoma* and *Piaractus* conducted in Work Plans 8, 9, and 10 and with new cultured species (*Prochilodus nigricans*, *Arapaima gigas*, *Brycon nigricans*, *Churo*, and *Congonpe*). Accordingly, we will:

- 1) Provide workshops to existing and prospective fish farmers in the Iquitos region. Specifically, we will update the Spanish language production manual for *Colossoma* and *Piaractus* compiled as part of Work Plan 10 to accompany the reproduction manual completed in Work Plan 9. These companion manuals will be used in workshops to be conducted at the IIAP Quistococha Aquaculture Station and at FONDEPES Iquitos for teaching prospective farmers the basics for pond culture. At least one workshop per year will be provided in Work Plan 11 to prospective fish farmers in the region in high schools and technical (vocational) schools. The latter workshops will be more general in nature and will serve as a primer for the more advanced workshops provided to existing producers. All workshops will include orientation on the business aspects of aquaculture.
- 2) Provide aquaculture advisement via site visits to local farmers. We will continue to make bi-monthly site visits to fish farms in the Iquitos area. Farms will be visited on a rotational basis so that every farm is visited at least once each quarter. Farmers will be provided with information on fish husbandry and pond maintenance, as well as with any new developments learned through our research activities. Standard water quality parameters (temperature, dissolved oxygen, pH, total ammonia-nitrogen, and nitrite) will be measured at representative farms throughout the region.
- 3) Evaluate the extension service through a questionnaire pilot tested and administered by the extensionists themselves to all clientele receiving extension services to assess quality of

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extension provided and to obtain suggestions on how to improve the program.

### Objective 2: Conduct demonstration projects with local fish farmers to expose them to new species and/or techniques.

Two successful techniques have been selected to expose local fish farmers and prospective fish farmers to successful productive techniques. The techniques consist of:

- 1) The training and certification of “Master Aquaculturists” and
- 2) On-farm research and demonstration projects.

In Work Plan 10, two successful producers were selected for training as “Master Aquaculturists.” A minimum of two additional producers will be selected for certification in Work Plan 11. CRSP personnel will continue working intensively with these producers to enhance their techniques and production efficiency. Once these farmers reach satisfactory levels, they will be certified as “Master Aquaculturists” and serve as mentors for novice farmers. The CRSP personnel will arrange for farm tours of these facilities, which will serve as living laboratories. Currently, in Work Plan 10, the CRSP has been experimenting with the use of mentors to transfer technology. We plan to build on this groundwork, for it appears to be working well.

The second technique will employ transferring technology to area fish farmers by conducting on-farm research and demonstration projects. This component will be achieved by conducting on-farm research as part of undergraduate student (UNAP) thesis projects, as has been successfully accomplished in Work Plan 10 (Alcántara, personal communication).

### Objective 3: Provide a short course to governmental and NGO personnel to develop a network of aquaculture extensionists in Peru and in neighboring countries.

One intensive training course for a small group of governmental and non-governmental personnel conducting aquaculture research and/or extension activities in the Amazon Basin will be offered at IIAP, Iquitos, Peru. This training plan will continue with the very successful program that has so far trained over 40 extensionists from Bolivia, Brazil, Colombia, Ecuador, and Peru. For each course, ten qualified candidates from Peru’s neighboring Amazon countries will be invited to participate, as well as a similar number from Peru. The course will be offered to train aquaculturists and experts in aquaculture related degrees in extension techniques. Techniques which have been practiced successfully by IIAP and Terra Nuova, including CRSP’s new experiences in the region through Work Plan 10. Extension personnel will also learn pond construction, broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, disease prevention and treatment, all specifically related to native cultured species of *Colossoma* sp., *Piaractus* sp., *Arapaima* sp., *Prochilodus* sp. and *Brycon* sp. (fish), and *Congompe* and *Churo* (mollusks) and to teach hormone injection, spawning, fertilization, incubation and larviculture techniques. A CD-ROM displaying all the course material for the Amazon aquaculture training course will also be produced to complement the written manuals.

### Objective 4: Develop an exchange program for researchers and students in the Amazon region.

An exchange program for ten university students and seven experts from several countries of the Amazon region will be designed. The prospective exchange students will be invited to participate in an academic exchange program to conduct either thesis work (three to six months) or short site visits (one week) to the aquaculture installations of neighboring countries, or to participate in seminars, workshops and symposiums covering Amazon aquaculture related topics. A fellow exchange student’s family in the “reciprocal” exchange country will provide for the food and lodging of the exchange student. The experts exchange program will cover the travel expenses and a small stipend to facilitate the exchange of experts for short periods (one to three days) from countries of the Amazon region to visit aquaculture sites and/or research organizations.

### Objective 5: Maintain and expand the specialized website on Amazonian aquaculture and species to provide for information exchange and networking.

A web site (<http://ws1.coopfish.siu.edu/amazonia/index.html>) on Amazonian aquaculture and

species will be maintained and expanded to allow for information exchange and networking. The web site will contain information on all CRSP-sponsored research and outreach activities in the Amazon region. It will also provide links to other agency activities in the region such as USAID, World Wildlife Fund, etc. An "AquaForum" will allow for discussions on Amazonian aquaculture and species by interested participants. The web site will contain a specialized bibliography on publications on research and outreach activities related to Amazonian aquaculture and species. An up-to-date list of announcements concerning related workshops and meetings will be maintained on the site. A list-serve will be established and maintained for the purpose of relaying relevant information on Amazonian aquaculture and species. The number of hits to the site will be enumerated to determine the site's exposure.

### **Regional and Global Integration**

An objective of the Regional Plan is to maintain and expand outreach and networking activities in the Amazon region. This proposal expands on this objective by training more personnel in neighboring countries as well as enhanced training in Iquitos. The proposal begins to build the network of mentors by certifying "Master Aquaculturists." It extends knowledge through on-farm trials and demonstration research. Lastly, the website on Amazonian aquaculture will facilitate networking both within and outside of the region.

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### Tilapia (*Oreochromis niloticus*) Production Constraints in Bangladesh

Sustainable Development and Food Security 2 (11SDFR2)/Activity/Bangladesh

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#### Objectives

- 1) Determine specific set of constraints impeding tilapia culture in Bangladesh.
- 2) Develop research priorities and strategies in order to remove obstacles facing seed production and/or production technologies of tilapia.
- 3) Organize a national level workshop and bring together all stakeholders to an open discussion on the present status as well as future potential and directions for tilapia production in Bangladesh.

#### Significance

Tilapia reaches market size (e.g., 100 to 150 g, preferred size for household consumption) within four months without feeding (Hussain, 1989). This allows for a minimum of two crops per year, making this an ideal species for culture in Bangladesh. The current stock of Nile tilapia, *Oreochromis niloticus*, was first introduced to Bangladesh by UNICEF in 1974, and later in 1987 by the Bangladesh Fisheries Research Institute (BFRI) from Thailand (Gupta et al., 1992).

The culture of tilapia is being promoted as a poor farmer's fish as well as one with export potential in many parts of Asia. An exception to this is Bangladesh. Despite early introduction and several favorable conditions for tilapia culture of Bangladesh, its production has not taken off. After nearly two decades of effort in breeding and culture of tilapia at BFRI and the Faculty of Fisheries, BAU (Hussain et al., 1989; Hussain et al., 2000), uptake of this technology by rural farmers and commercial entrepreneurs has been extremely slow. Even the Department of Fisheries extension agency (which has many graduates from AIT with training in tilapia culture) has not been able to provide tilapia culture technology from well-developed systems in neighboring countries (e.g., the Philippines, Thailand, and Vietnam). There could be a number of reasons for this including the lack of institutional support, knowledge on suitable culture technology, seed, lack of trained personnel, markets, weakness in the promotion policies, and incompatibility of this fish in the Bangladesh culture systems.

A comprehensive study is needed to determine what the constraints are (technical, human resource, institutional, and/or market) and then to set priorities and develop research and production strategies necessary to facilitate production of tilapia. Unless steps are taken to resolve existing constraints in culture of tilapia, full potential of this valuable species as food for the poor will not be realized in Bangladesh.

#### Anticipated Benefits

Successful completion of this study will elucidate:

- 1) Technical, human resource, or market constraints associated with tilapia production in Bangladesh.



- 2) Research needs related to tilapia production in Bangladesh, which will facilitate prioritizing and formulating proposals for CRSP funding for the Twelfth Work Plan.

## **Research Design**

*Location:* Bangladesh Agricultural University, Mymensingh, Bangladesh.

*Survey Design and Procedures:* A set of questionnaires will be developed for institutions involved in tilapia research (government, nongovernment, private, research institutes, and universities) to determine the current information base. Publications in the scientific journals, newspapers, magazines, and books in Bangladesh regarding the tilapia culture will be collected, reviewed, and cataloged for future use.

All seed production hatcheries for tilapia as well as 10% of representative carp hatcheries will be visited, and production techniques, capacity, present need, and supply will be assessed (observation and personal interviews). The broodstock and their management (if any) will be identified, and distribution channels in the country will also be identified. Farmers involved in tilapia farming and entrepreneurs interested in this species will be identified and interviewed on various aspects including marketing and distribution, preservation, and the quality control and value they get versus their cost will be evaluated. The extension agents and others who have received training from academic institutions will be identified, and views on this species and its prospects of promotion will be gathered.

Finally, a national-level workshop bringing all relevant people including the nongovernmental organizations, private entrepreneurs, and government policy makers to share the findings of the study and to discuss ways to address constraints through science-based research. Efforts will also be made to invite some relevant people from other south Asian countries (Nepal, Sri Lanka, and Pakistan) where tilapia production issues are similar to Bangladesh.

*Analysis:* A statistical analysis is not appropriate for this study.

## **Regional Integration**

Successful completion of this survey will be communicated to other South Asian (Nepal, Bhutan, and Sri Lanka) countries through our Asian Institute of Technology channels. The planned workshop will involve integration of regional institutions.

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### Reproductive Performance and Growth of Improved Tilapia, *Oreochromis niloticus*

Sustainable Development and Food Security 3 (11SDFR3)/Experiment/Thailand

#### Investigators

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Graham C. Mair	HC Co-Principal Investigator	Asian Institute of Technology, Thailand
James S. Diana	US Principal Investigator	The University of Michigan

#### Objectives

- 1) Compare reproductive performance (fecundity, spawning frequency, fertilization, hatch, and larval survival) of three improved strains (GIFT, IDRC, and Fishgen) and the Thai Chitralada strain of Nile tilapia.
- 2) Compare the growth rates and age and size at sexual maturation of the four strains in earthen ponds and in an intensive recirculation system.

#### Significance

The CRSP studies have made contributions to determining the optimal fertilization regimes for various locations (Brown et al., 2001; Veverica et al., 2000), feeding and feed types (Diana et al., 1996), production of monosex population (Green and Teichert-Coddington, 1994; Phelps and Warrington, 2001; Gale et al., 1999), and polyculture with other species (Syzper and Hopkins, 1997). AIT has also carried out studies on the development and optimization of hapa-based breeding systems for tilapia—systems now adopted widely through out Asia. These and other studies have added significantly to our understanding of tilapia biology and the environment in which they are raised. Neither the CRSP nor AIT has had any significant involvement in the improvement of tilapia for aquaculture.

A number of important and successful breeding programs have been and are being carried out for tilapia, mostly in the Philippines. These studies have received attention primarily because of farmer perception that earlier domesticated stock growth rates and reproductive performance have declined over many generations of domestication and local adaptation. ICLARM's GIFT (Genetically Improved Farmed Tilapia) project demonstrated, through its breeding program based on genetically diverse germ plasm from Africa and Asia, that significant gains in production can be achieved by selective breeding. Estimates of genetic gain for the later generations of the selected GIFT strain are as high as 13% per generation over five generations providing an estimated cumulative increase of 85% in growth rate compared to the base population from which it was selected (Eknath and Acosta, 1998). These stocks have now been distributed to fisheries agencies throughout the south and southeast Asian tilapia-producing countries. There have been a number of other successful breeding programs carried out for tilapia in several countries including the Philippines, Thailand, and Vietnam. Additionally, the YY male breeding program for the production of Genetically Male Tilapia (GMT) is also now widely distributed throughout the region (Mair et al., 1997).

Currently there are at least three strains in Asia in which significant improvement in growth rate has been achieved:

- 1) GIFT has undergone seven generations of combined selection (Eknath and Acosta, 1998);
- 2) IDRC strain has undergone 13 generations of within-family selection (Bolívar and Newkirk, 2000); and
- 3) Fishgen-selected strain has undergone three generations of intensive selection on a stock of combined superior strains (Abucay and Mair, 2000).

All these strains are now being bred at AIT. In addition the Thai Chitralada strain has developed a reputation throughout the region and beyond for its superior growth performance traits, despite not having been subject to any deliberate improvement efforts (Yakupitiyage, 1998). Although the selected



lines are thought to have higher growth rates, there has been little independent verification of the gains nor any comparison of the improved stocks with each other. In addition, there may have been a number of correlated responses to selection that could impact upon other traits, such as reproduction, that have not been subject to comparative evaluation. While the primary interest from tilapia producers is in the growth rates of these strains, hatchery managers need to know the reproductive properties of these strains in order to manage them effectively. Some strains may have correlated traits, such as late maturation, which are undesirable to seed producers and would severely limit their distribution despite having attractive properties to growers. Given the interest in and demand for these improved breeds, their potential to boost yields and economic benefits in low-input farming systems, and the fact that they are already being widely disseminated, there is a need to quantify the comparative reproductive capacity (as one of the most important of correlated traits). This study, therefore, proposes to compare reproductive performances and growth of improved tilapia (e.g., GIFT, IDRC, Fishgen) and simultaneously compare them with Chitralada strain at AIT.

## Anticipated Benefits

Successful completion of this study will:

- 1) Enhance understanding of the comparative reproductive potential of the improved strains as a trait correlated to growth and thus probably modified by selection;
- 2) Provide evidence to substantiate the respective claims for improved growth of the three improved varieties under at least two environmental conditions; and
- 3) Allow the formulation of recommendations for the appropriate use of one or more select groups over the others based on reproductive performance, growth, and the interrelationship between these traits in different environments.

## Research Design

*Location:* AIT, Thailand.

*Methods:* Experimental fish—The GIFT (seventh generation select), Fishgen (third generation select), IDRC (thirteenth generation select), and the Thai Chitralada strains—are held and will be bred at AIT. Broodfish will be held in hapas (5 m<sup>3</sup>) in 335 m<sup>2</sup> surface area ponds. A fertilization and feeding regime will be followed based on previous CRSP studies. For monosex production, fry will be subject to hormonal sex reversal using standard methods of oral application of 17 $\alpha$ -methyltestosterone.

### Experiment 1. Reproductive Performance of Four Different Broodstocks of Tilapia—GIFT, IDRC, Fishgen, and Chitralada at AIT

*Pond and Hapa Facilities:* One earthen pond (335 m<sup>2</sup> surface area) and 12 hapas (5 m<sup>3</sup> each) will be used to hold the broodfish with three replicate hapas per strain. Water depth will be maintained at 1.2 m in the pond and 1.0 m in the hapa leaving a 20 cm space between the pond and the hapa bottom.

*Stocking Density:* Twenty 15-month-old fish will be stocked per hapa (5 males to 15 females). All spawned females will be individually marked with PIT tags.

*Fertilization and Feeding Regime:* Ponds will be fertilized using 4N:1P and addition of urea or TSP if required.

*Sampling:* Eggs from females will be collected at seven-day intervals, when their stage of development will be determined, and then incubated using standard tray systems (Little, 1989) in the AIT hatchery. The following data will be collected over a 12-month period:

- Spawning frequency of individual females in each group.
- Number of eggs per stage per female (which will be identified and weighed).
- Hatching rate of eggs for each stage collected for each group.
- Survival of fry, 15 days post-hatch.

In addition visual assessment of general conditions of the broodfish, eggs, sperm, embryos, and

## ELEVENTH WORK PLAN

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larvae will be made to compare with observed numbers.

### Experiment 2. Comparisons of Growth Rates and Age/Size at Sexual Maturation between the Four Strains of Tilapia in Two Culture Systems (Extensive-Fertilization Only in Ponds and Intensive in Recirculating Tank Systems) under Mixed-Sex and Monosex Sex Ratio “Environments”

Comparisons will be done under communal stocking of the four strains in mixed-sex and monosex sex ratio “environments” (4 × 200<sup>2</sup> m pond for each sex ratio environment). The fertilization-only pond systems will represent the slow growth systems. Similar comparisons will be made in intensive systems where fish would be expected to grow very fast. Comparisons in intensive systems will thus likewise be done under communal stocking of the four strains in mixed-sex and monosex sex ratio “environments” (4 × 3.6 m<sup>2</sup> concrete tanks within a recirculating system for each sex ratio environment). All fish will be stocked at the same age, at a mean weight of 5 to 10 g, and marked with a combination of coded wire tag and fin clipping.

*Pond Facility:* Eight earthen ponds of 200 m<sup>2</sup> surface area will be used, four for each sex ratio “environment.” We will maintain water depth of 1.0 m in 1.2 m deep ponds.

*Tank Facility:* Eight circular concrete tanks (3.6 m<sup>2</sup>) within the same recirculating system will be used, four for each sex ratio “environment.”

*Culture Period:* 250 days.

*Base Stocking Densities:* 2.7 fish per m<sup>2</sup> in open ponds; 640 fish (5 to 10 g per fish) per tank (160 fish per strain) reduced to 80 fish per tank (20 fish per strain) for the final grow-out. The stock will be reduced in number by sampling for assessment of sexual maturation in both systems.

*Nutrient Input:* Fertilize ponds at a rate of 4N:1P for the extensive system. Feed pelleted feeds at 5, 3.5 and 2.5% body weight per day during the first, second, and third stages of grow-out for the intensive system.

*Sampling:* Water quality: Standard CRSP protocol. Fish growth will be sampled biweekly. To assess the rate of gonad development and time of sexual maturation, ten males and ten females will be removed from the pond and sacrificed every 21 days during the period of sexual maturation (three to seven months of age). Initial stocking rates will be calculated to compensate for reduction in density caused by this sampling.

#### *Experimental Design, Null hypothesis, and Statistical Analyses:*

Experiment 1: Four treatments (four strains of tilapia) will be compared in replicates of three breeding hapas per strain. Variables measured include spawning, spawning frequency, fecundity, hatching rate, and survival rate of fry.

- Null hypothesis: There is no difference between the four strains of tilapia in relation to reproductive performance over time.
- Statistical analyses: Data will be summarized in data tables and figures. Significant difference between four strain means will be analyzed using ANOVA. If treatment differences are observed, analysis of least significant difference will be used at  $\alpha = 0.05$  level of significance.

Experiment 2: Growth means of four strains in two culture systems (extensive pond and intensive tank) and sex ratio “environments” (monosex and mixed sex) will be compared. Weight and length of fish will be measured at 21-day interval samplings at which point some fish will also be sampled and sacrificed for assessing sexual maturation starting at three months of age.

- Null hypotheses: There are no differences in growth rates, age, and size at sexual maturation between the four strains of fish tested in the two different culture systems. There is no difference in the relative growth of the strain in mixed-sex and monosex “environments.”  
An overall null hypothesis: There is no genotype-environment interaction in the relative growth of the four strains.

- Statistical analyses: Data will be summarized in data tables and figures. Significant difference between four different strain means, two systems, and two sex ratio “environments” will be analyzed using t-test (within culture units), ANOVA, and regression. If treatment differences are observed, pair comparisons will be made using LSD ( $\alpha = 0.05$ ). Individual fish will serve as replicates within culture units (i.e., pond or tank), and there will be four replicates of the units per system, per sex ratio environment. Data from units can be pooled if not significantly different.

### **Regional Integration**

Locally available tilapia in the region is seen as a poor performer in many countries including the Philippines, Laos, Vietnam, and Bangladesh. Results of this study will identify and verify the best performing tilapia, which are likely to be quickly adopted in the above and other tilapia producing countries.

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### Diversification Into Sustainable Tilapia-Shrimp Polyculture and Small-Scale Tilapia Cage Culture in Mexico

Production System Design and Integration 1 (11PSDR1)/Activity/Mexico

#### Investigators

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#### Objectives

The objectives of this project are firstly to identify and analyze the factors that are adversely affecting shrimp farming in northwest Mexico and secondly to consider the sustainability of tilapia-shrimp polyculture as an alternative to shrimp monoculture.

Specifically, the research team will:

- 1) Develop a study to determine which factors are adversely affecting the shrimp farming industry resulting in a proportion of farms not operating and a further proportion not operating at full capacity. The study should identify if a common factor exists between farms with operational problems. Lastly, the study would consider if these factors are unique to the shrimp farming industry or could also affect tilapia-shrimp polyculture.
- 2) Develop a study to determine the sustainability of tilapia-shrimp polyculture. The study would incorporate a cost analysis of tilapia-shrimp polyculture to determine production costs and therefore production levels required to make a profit under environmental and economic conditions in Mexico. The study would also consider the possible groups that may take up such a culture and the identified factors adversely affecting the shrimp farming industry that may affect tilapia-shrimp polyculture.

#### Introduction

Disease outbreaks and price fluctuations continue to affect the stability of the shrimp industry in many parts of the world. The results from the surveys of shrimp and tilapia farming (PD/A CRSP project, 10NSR3A-E) in Thailand, the Philippines, and Mexico show that shrimp ponds have been abandoned due to diseases, poor management, and environmental degradation. The project's surveys and experiments also support the suggestion that tilapia production, supplemented with low densities of shrimp, in shrimp ponds can provide an opportunity to develop a sustainable aquaculture system (Fitzsimmons, 2001) that will support local inhabitants who have been failed by shrimp aquaculture or who did not benefit from the shrimp farming boom. Tilapia production in former shrimp ponds (with and without shrimp) has increased rapidly in many of the PD/A CRSP locations including Thailand, the Philippines, Honduras, Peru, and the inland desert of Arizona.

The survey conducted in Mexico shows that 76% of the shrimp farms experienced production problems and many of these farms are considering alternative aquaculture species as an opportunity to stabilize production. Tilapia culture in shrimp ponds is being considered by 53% of the farmers. The main two constraints in Mexico for the development of tilapia as an alternative species for culture in shrimp ponds are knowledge of the biotechnologies required for culture in seawater and supply of seed of salinity-tolerant strains of tilapia. This is a unique opportunity to continue harnessing the strengths of the PD/A CRSPs expertise and aid the development of a sustainable aquaculture system that would both safeguard the jobs of many local inhabitants and provide work to low-income fishermen in coastal areas. This proposal would continue the work initiated between researchers and industry partners and would initiate work with low-income fishermen. The anticipated benefits of the research are: a production system that is more sustainable than the current shrimp farming system, a cage production system for use by fishing communities, an increased opportunity for local inhabitants to have reliable job opportunities, and supplemental income from tilapia culture for low-income fishermen.

Shrimp aquaculture in Mexico is in crisis due to a mix of the depressed world shrimp market and disease outbreaks causing decreased yields (Panorama Acuicola, 2002). The PD/A CRSP survey showed that the main cause of low yields was the viral disease white spot syndrome, which accounted for 59% of the reported problems causing low production. All these problems were in recent years; white spot syndrome was first diagnosed in Mexico in 1999. These low yields due to disease outbreaks, combined with a low world price for shrimp, have pushed many operations into a situation where they are no longer profitable.

The majority of the Mexican shrimp farming industry is situated in the northwest of Mexico (SAGARPA CONAPESCA, 2000). Aquaculture production of shrimp in Mexico has developed rapidly from 35 tons in 1985 to 33,481 tons in 2000 (SAGARPA CONAPESCA, 2000). The industry directly employs 23,505 and many more indirectly in secondary industries that supply feeds and equipment, and process and export the final product. A large number of these employees are local people, often employed in the poorer paid menial jobs and a proportion of the positions in middle management. These people have also benefited from improved infrastructure (roads and electrification) that has come with the shrimp farms and general development of these areas. The industry's development has been regulated through a mixture of government intervention and the complications of coastal land tenure that has restricted the speed of growth compared to other shrimp producing countries. Although cases exist of salination of agricultural land and destruction of mangrove and artisanal fisheries, the development of shrimp aquaculture in Mexico has not caused widespread environmental problems (Hernández-Cornejo and Ruiz-Luna, 2000).

Basically three groups are involved in the industry: international companies, national companies, and social cooperatives. At present a large proportion of the industry is operating on credit or profits from past years. The majority of producers are not being paid until the processor sells the shrimp. In turn the hatcheries supplying larvae and feed suppliers are not being paid until harvest or when the processor pays the producer. The international and national companies are at present managing in this situation. Both have financial reserves from past years, but clearly the international companies will be considering pulling out of Mexico while national companies must continue or diversify into other areas. At present the social cooperatives are the least prepared for this situation—past profits were often distributed between many hands and little reserve exists to deal with a no-profit situation.

Production procedures are changing rapidly to deal with these disease outbreaks and low yields (Roque and Gómez-Gil, 2000). Many farms have reduced costs by reducing stocking densities and the number of cycles of shrimp production in a year. However, clearly this also reduces production. The majority of the disease outbreaks have been observed during the rainy season (July to October). The rains cause large environmental fluctuations in salinity, temperature, and turbidity. It is suspected that these environmental fluctuations stress the shrimps and trigger disease outbreaks. In the past one or two years, a number of farmers operated for just one cycle, stocking at low densities after the rainy season (December to February) and harvesting before the start of the rainy season (May to July). This is a longer production cycle, and larger shrimp were harvested giving good yields per hectare. Although this system enables these farms to continue operating, they are not fully utilizing the shrimp ponds, which are being abandoned for a part of the year. This results in a seasonal job market for many of the local people or even the people operating the social cooperative farms. It is considered that tilapia would be well suited to culture during this part of the year when ponds are not being used. Lower salinities associated with the rain would favor tilapia culture. Tilapia and low densities of shrimp could be stocked at the start of the rainy season in lower salinities and cultured through to December for Christmas markets. The combination of one shrimp cycle and one cycle of tilapia-shrimp polyculture using the culture system developed in collaboration between researchers and industry in the CRSP project would help the social cooperatives operate throughout the year giving a higher financial return from the infrastructure and providing fuller employment for the cooperatives and other local people.

Many of these local people come from fishing communities. Many of these communities have not benefited from developments in coastal aquaculture and continue to earn a living from artisanal coastal fisheries. In recent years catches for these communities have been declining or, at best, variable—



offering both good and bad years. These communities are becoming marginalized, often abandoning their homes and boats to seek employment in nearby cities during periods of poor catch or low season. There are 99,804 small artisanal fishing boats registered in Mexico (SAGARPA CONAPESCA, 2000) indicting the extent of this problem. These communities are often situated in sheltered bays or estuaries that are suitable for small-scale cage culture. On-growing of commercially important species, such as tilapia, in small-scale cage culture is an activity that is viable for these communities. The cage culture of seawater strains of tilapia would provide an alternative sustainable income for these people.

### Ecological Basis for Tilapia-Shrimp Polyculture

In nature, tilapia are omnivores. Young tilapia graze on algal and bacterial films, scraping most hard surfaces with tongue and teeth. As they grow they also become effective filter feeders of phytoplankton and predators of zooplankton. Larger tilapia are less effective filter feeders but begin to graze heavily on macrophytic algae and aquatic plants. In extensive farming situations, tilapia filter feed on algae, prey on zooplankton, and scrape films from any hard surfaces in the pond. In intensive farms, most nutrition is derived from pelleted feeds, although fish will continue to spend time scraping algal and bacterial films from all surfaces.

In nature, shrimp feed first on phytoplankton and then zooplankton during larval stages. As juveniles and adults, they are omnivores and detritivores. Their natural behavior is to search the bottom substrates for decaying plant and animal material. They also constantly pick up sand grains and pieces of organic matter and graze off the algae and bacteria, drop the grain or particle, and go onto the next item. In farmed settings shrimp feed on pellets and natural productivity in the pond. Research by Samocha et al. (1998) has demonstrated that shrimp can be reared in systems with little water exchange, taking advantage of the natural abilities of shrimp to thrive in conditions with high bacterial loading so long as dissolved oxygen (DO) levels and other water quality factors are maintained.

There are several variations of tilapia-shrimp polycultures: simultaneous, sequential, and crop rotation. In the simultaneous instance, the fish and shrimp are grown together in a pond or raceway; in the sequential case the water is moved from one growing unit to another; and the crop rotation alternates tilapia and shrimp. There appear to be distinct advantages with each of these systems.

In a polyculture setting, tilapia and shrimp can utilize different niches. In an extensive farm, tilapia can filter feed on phytoplankton and zooplankton in the upper water column. Shrimp spend most of the time in the pond bottom grazing on bacterial films on the bottom substrate and on the detritus settling from above. This detrital matter consists of dying algae cells and fecal matter from the tilapia. In a more intensive farm receiving pelleted feeds, the tilapia monopolize the feed, especially if it is a floating feed. However, some feed particles always get to the bottom where the shrimp will get it. More importantly, the fecal matter from the tilapia contributes to the detrital rain that supports the shrimp. *Macrobrachium*-tilapia polyculture reduces the yield of prawns compared to monoculture, but increases total yield of fish and prawns (Garcia-Perez et al., 2000). A similar effect occurs with brackishwater polyculture of tilapia and shrimp (Yap, 2001). Anggawa (1999) reported that yields of shrimp increased when tilapia were stocked into existing shrimp ponds. The suggested stocking rate was 20 to 25 g fish m<sup>-2</sup> and the fish size at stocking of 50 to 100 g per fish. The use of all-male fish was needed to control reproduction. Fish were stocked when the shrimp biomass was at least 80 g m<sup>-2</sup> (for 3-4 g shrimp) or 150 g m<sup>-2</sup> (for 5 to 6 g shrimp). Tilapia harvest biomass was 40 to 50 g m<sup>-2</sup>, and shrimp survival was 70%.

From the disease aspect, tilapia seem to provide advantages in several ways. Growers in Ecuador have reported that tilapia will consume dead or moribund shrimp in polycultured ponds. Cannibalism is one of the primary vectors for transmission of shrimp diseases. Tilapia, which do not appear to be susceptible to or carriers of these viruses, disrupt cannibalism as a mode of transmission. Tilapia also consume small crustaceans in shrimp ponds. These crustaceans are of concern as potential vectors. Having tilapia directly in the ponds or alternating with shrimp in a crop rotation can be effective for reducing crustacean populations. Bacterial infections also may be impacted by polyculture. *Vibrio* and most other bacterial pathogens common in shrimp culture are gram negative while waters which have

been used for fish culture tend to be predominated by gram positive bacteria. Using water from a fish culture pond seems to reduce the prevalence of luminous *Vibrio* bacterial infections in shrimp ponds (Yap, 2001). Growers in Asia and South America have provided anecdotal reports that shrimp production increases due to higher survival in some of these polyculture systems. However, carefully controlled and replicated trials are needed to better study these systems and confirm the results.

There may also be physical factors that improve shrimp survival and growth in polyculture and crop rotations. Tilapia disturb bottom sediments to a greater degree than shrimp, both in foraging and nest building activities. This may be beneficial in several ways. Disturbing the bottom could improve oxidation of the substrate and interrupt life cycles of shrimp pathogens and parasites. It could also release nutrients into the water column that could improve algae blooms. However, it is also possible that these activities may be detrimental. Disturbing bottom sediments could also negatively impact water quality by lowering DO levels, increasing turbidity from sediments, and reducing algae blooms, limiting ability to remove fish and shrimp, and most certainly increase the need to repair pond bottoms between crops. This particular aspect would require close attention and careful experimentation to gain a clear understanding.

### **Anticipated Benefits**

Two completed studies indicating possible problems and the potential of sustainable tilapia-shrimp polyculture. These studies would be disseminated to government bodies, industry groups, and farmers. To whom the reports were disseminated would be recorded. We would hope the studies would promote further action such as further analysis of polyculture, government or industry group action to promote polyculture, or the establishment of farms operating tilapia-shrimp polyculture. Any such actions would be recorded and quantified.

### **Activity Plan**

#### **Study of the Factors Adversely Affecting Shrimp Culture**

A wide cross section of the shrimp farming community would be interviewed for their opinion on the factors that are adversely affecting the shrimp farming industry. This group would include government personnel working to promote and regulate the industry, industry organizations such as the Sinaloa Institute of Aquaculture (Instituto Sinaloense de Acuicultura, ISA—an organization funded by producers and local government), shrimp farms from a range of backgrounds (international and national companies and cooperatives), and leaders in the processing and marketing industry. A concerted effort would be made to contact and interview present or previous owners of farms no longer operating. Available literature including a substantial body of “gray” literature (industry and government publications and university theses) would be reviewed. These opinions would be combined to draw conclusions on which factors are adversely affecting the shrimp farming industry resulting in a proportion of farms not operating and a further proportion not operating to full capacity and identify if a common factor exists between farms with operational problems. Lastly, Kevin Fitzsimmons, Neil Duncan, and the authors would consider if these factors are unique to the shrimp farming industry or could also affect tilapia-shrimp polyculture. This study would form an undergraduate thesis.

#### **Study of the Sustainability of Tilapia-Shrimp Polyculture in Mexico**

The study would consider the sustainability of tilapia-shrimp polyculture from an economic and environmental perspective. The principal component for the consideration of economic sustainability would be the cost analysis of tilapia-shrimp polyculture in Mexico. The cost analysis would consider a wide range of parameters that affect the economic outcome of a farming operation: stocking density, growth, feed costs, pumping costs, mortality rates, and maintenance and depreciation of infrastructure. This study would build a model of a single grow out cycle of tilapia-shrimp polyculture. The model would indicate the equilibrium points for the different parameters, suggesting what combinations of stocking densities and water exchange rates could be used to ensure a good economic return. From the environmental perspective, the adequacy of the environmental variables for seawater culture of tilapia would be compared with parameters from existing polyculture systems in other countries and expected farm discharges would be compared with shrimp

farm discharges. These considerations and the factors that adversely affect the shrimp farming industry would be discussed and recommendations made on the possible future of tilapia-shrimp polyculture in northwest Mexico. These results would form a graduate thesis and be published in the scientific literature, at regional and international aquaculture conferences, and be shared with others who would be interested to implement tilapia-shrimp polyculture.

### Regional Integration

Honduras and Panama are regional producers who could also benefit from tilapia-shrimp polyculture and studies to evaluate the potential of these cultures. Tilapia and shrimp production are growing in the border area of Mexico and the US. Further coordination between US and Mexican scientists and producers is needed. Sharing of graduate students and cooperation on regional conferences are desired goals to improve integration. Through regional aquaculture meetings in Honduras and Mexico, and development of e-mail and websites, we expect to further integrate aquaculture findings and developments.

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## New Paradigm in Farming of Freshwater Prawn (*Macrobrachium rosenbergii*) with Closed and Recycle Systems

Production System Design and Integration 2 (11PSDR2)/Experiment/Thailand

### Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology, Pathumthani, Thailand
James S. Diana	US Principal Investigator	The University of Michigan
C. Kwei Lin	US Co-Principal Investigator	The University of Michigan

### Objectives

- 1) Develop closed and recycle systems for prawn culture.
- 2) Assess economic and environmental impacts of those new systems.

### Significance

The freshwater prawn (*Macrobrachium rosenbergii*) is indigenous to most southeast Asian and south Pacific countries (New, 1982). Since its successful domestication in the late 1960s (Ling, 1969), the culture of freshwater prawn has gained great popularity worldwide, mostly in tropical and subtropical regions, with limited production in temperate regions such as North America (D'Abramo et al., 1989). In recent years the global production of freshwater prawn has increased steadily (FAO, 1996) with major production in east and south Asian countries—China, India, Indonesia, Bangladesh, Thailand, and the Philippines. Consisting primarily of *Macrobrachium*, freshwater crustacean production in the region reached 0.5 million tonnes (FAO, 1998). With FAO's efforts on development of freshwater prawn culture, a center for research and training was established in Thailand to serve southeast Asia in the 1970s and 1980s. A number of production systems were developed over the years including primarily pond, pen, and paddy culture. By far the most prevalent production system has been intensive pond culture. The widely practiced standard technologies for pond culture involve stocking with hatchery seed, feeding with formulated diets, and maintaining water quality by frequent exchanges (New and Singholka, 1985). On average, annual production reaches 2,000 kg per cycle. New (1991) also provided a review on prawn farming.

Despite the expansion of prawn culture for several decades, few changes have taken place in culture technologies. Under the present intensive operation, prawns are fed with a formulated protein-rich diet and as a result the pond water deteriorates rapidly. Mechanical aeration is rarely used in ponds. Frequent water exchange with an external water source is required to maintain pond water quality. This open system resembles intensive marine shrimp culture that causes serious environmental problems, making production unsustainable. Currently, prawn farms discharge nutrient-rich organic effluent to public waterways resulting in profuse growth of aquatic weeds as well as making those waters unsuitable for other uses. In Thailand, for instance, most prawn farms are located along irrigation canals that supply water to and receive discharge from ponds. As canals serve multiple users, waters are often contaminated with domestic and agricultural wastes. Prawn culture technologies should be improved to mitigate these environmental problems. Presently, the culture systems used to produce prawns are not well understood.

Wastewater from intensive fish culture has been shown to be effective for producing phytoplankton to support Nile tilapia (*Oreochromis niloticus*) culture (Lin et al., 1990; Lin and Diana, 1995; Yi et al., 2001). Apparently benefiting from the nutrient rich effluents, aquatic macrophytes also grow profusely in the areas where effluents are discharged. Water mimosa (*Neptunia oleracea*) and water chestnut (*Trapa bispinosa*) are widely cultivated in fertilized tanks and ponds and harvested for human consumption in the region. To mitigate the problems of wastewater discharge that pollutes surrounding areas, we propose a new closed system with circulators whereby effluents from freshwater prawn ponds could be recirculated for culture of tilapia, water mimosa, and water chestnut. Such diversification and

## ELEVENTH WORK PLAN

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integration are regarded as important practices to enhance sustainable aquaculture (Alder et al., 1996; Pillay, 1996). We will also survey existing farms to better understand current production systems.

### Anticipated Benefits

The closed recycle culture system for prawn farming will advance culture technologies toward a more sustainable paradigm by reducing environmental contamination to public waterways and reusing the waste for other crops. This new approach is important not only to increase farmers' awareness of environmental problems but also to provide new means to improve the system. Successful demonstration of this research in Thailand will serve as a future model for prawn farming in the region.

### Research Design

*Location:* AIT, Thailand.

*Methods:* Pond experiments at AIT.

*Pond Facility:* 15 earthen ponds of 300 m<sup>2</sup> in surface area, 0.8 to 1.2 m water depth.

*Culture Period:* Eight months.

*Stocking Density:* 10 prawn per m<sup>2</sup>; 2 tilapia per m<sup>2</sup>.

*Test Species:* Freshwater prawn (*Macrobrachium rosenbergii*); Nile tilapia (*Oreochromis niloticus*); water mimosa (*Neptunia oleracea*); water chestnut (*Trapa bispinosa*).

*Nutrient Inputs:* Commercial pelleted feed for prawn.

#### *Sampling Schedule:*

Water quality: Standard CRSP protocol, biweekly water quality sampling, and monthly diel analysis at various depths.

Animal/plant growth: Biweekly and total harvests.

Soil samples: At beginning and end of the experiment for moisture, bulk density, TN, and TP.

Feed: Monthly analyses for moisture, TN, and TP.

Nutrient budgets will be determined.

Partial financial budgets will be analyzed to assess costs and values of prawn, tilapia, and macrophyte crops.

#### *Statistical Design, Null Hypothesis, and Statistical Analysis:*

There will be three treatments with three replicates each:

- 1) Traditional open system: Prawn pond alone of 0.8 m deep; water exchange with external water at rate of 5 to 30% weekly.
- 2) Closed system: Prawn pond alone of 1.5 m deep; no water exchange; mixing pond water with a circulator daily between 1400 to 1600 h.
- 3) Recycle system: Consisting of three ponds; one for prawn (1.2 m deep), one for tilapia (1.0 m deep), and one for macrophytes (0.8 m deep); circulating water from prawn pond to tilapia pond and then to macrophyte pond by a circulator in prawn pond and recycled through overflow pipes to other pond.

Null hypothesis: There are no differences in prawn growth, water quality, effluent quality, and nutrient utilization among treatments.

The results of prawn growth, water quality, effluent, and nutrient utilization will be analyzed for significant differences among treatments using ANOVA.

## Regional Integration

Freshwater prawn, Nile tilapia, water mimosa, and water chestnut are widely cultivated in the region. The closed and recycle systems will be a new step in production technology that will promote efficient production as well as environmental sustainability.

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### Integrated Cage-Cum-Pond Culture Systems with High-Valued Fish Species in Cages and Low-Valued Species in Open Ponds

Production System Design and Integration 3 (11PSDR3)/Activity/  
Thailand, Bangladesh, Nepal, Vietnam

#### Investigators

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C. Kwei Lin	US Co-Principal Investigator	The University of Michigan

#### Objectives

- 1) Adapt the integrated cage-cum-pond systems developed by the PD/A CRSP to local conditions.
- 2) Determine appropriate stocking density of selected fish species in cages.
- 3) Assess growth and production of fishes in both cages and open ponds.
- 4) Assess the economic and environmental benefits of this integrated system.

#### Significance

The integrated cage-cum-pond culture system is a system in which high-valued fish species are fed with artificial diets in cages suspended in ponds, where filter-feeding fish species are stocked to utilize natural foods derived from cage wastes. This integrated system has been developed and practiced using combinations of catfish-tilapia (Lin, 1990; Lin and Diana, 1995) and tilapia-tilapia (Yi et al., 1996; Yi, 1997; Yi and Lin, 2000, 2001) at AIT. Although cages were set up in Nile tilapia monoculture ponds in all previous work mentioned above, this integrated system can be applied in polyculture systems. In polyculture, ponds are stocked with several species of different feeding habits together. It is impossible to target feeding to only high-valued species because low-valued species consume the feed resulting in economic inefficiency unless an integrated system is adopted. Compared to the nutrient utilization efficiency of about 30% in most intensive culture systems (Beveridge and Phillips, 1993; Acosta-Nassar et al., 1994), the nutrient utilization efficiency could reach more than 50% in integrated cage-cum-pond systems, resulting in the release of much less nutrients to the surrounding environment (Yi, 1997).

Rural pond aquaculture in Nepal, Bangladesh, and Vietnam is mainly the semi-intensive carp polyculture of both Indian major and Chinese carps with low production (for example, 2.8 tonnes per hectare in Bangladesh; Department of Fisheries, 2001). Pond production systems in many countries are becoming increasingly reliant on external resources (feed and/or fertilizers) to supplement or stimulate autochthonous food production for fish. Such a system often discourages small-scale poor farmers because of low return on investment. On the other hand, such poor farmers have limited financial resources to turn their whole ponds to culture high-valued species using expensive artificial feed. However, the integrated cage-cum-pond system provides an opportunity for small-scale farmers to use their limited resources to include a small amount of high-valued species in their ponds to generate more income and improve their livelihood. This is achieved through improved nutrient utilization efficiency, marketing high-valued species, and saving fertilizer cost because fish in open water can efficiently utilize cage wastes and there is no fertilization required. Also, this integrated cage-cum-pond system is environmentally friendly due to less waste nutrients released to the environment.

The proposed work on the integrated cage-cum-pond system will be conducted on-station and on-farm in Thailand, Bangladesh, Nepal, and Vietnam. Important high-valued indigenous species in each country will be used to stock cages, including stinging catfish (*Heteropneustes fossilis*) in Bangladesh,

sahar or mahseer fish (*Tor putitora*) in Nepal, and climbing perch (*Anabas testudineus*) in Vietnam. Sahar culture in ponds is not very successful, and Islam (2002) concluded that this species is not suitable in pond monoculture due to extremely high FCR (5 to 7). The integrated cage-cum-pond may be suitable to culture this species. All other species proposed above are air-breathing and thus can be cultured in cages at high densities. For the on-farm trial, school ponds in Thailand will be included by stocking hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) in cages. There are more than 2,000 ponds in schools in Thailand, which are supposed to be used to provide free high-protein lunches for students especially from poor families, however, they have not been well used. This integrated cage-cum-pond system may be appropriate for school ponds, and it is particularly important to increase students' awareness of environmental problems and means to minimize environmental pollution while enjoying fun of feeding caged fish.

## **Anticipated Benefits**

This technology will provide small-scale rural farmers an opportunity to generate more income and improve their livelihood using their scarce resources and will benefit small-scale rural farmers in Asian and other countries where integrated systems are practiced.

## **Research Design**

*Location:* Bangladesh, Nepal, Thailand, and Vietnam.

*Methods:* Pond research at BAU, IAAS, and CTU.

*Pond Facility:* Fifteen 100 or 200 m<sup>2</sup> earthen ponds at each institution.

*Cage Facility:* Twelve 4.0 m<sup>3</sup> cages at each institution.

*Culture Period:* 150 days.

*Test Species:*

For open ponds: Site specific based on local practices.

For cages: Indigenous stinging catfish (*Heteropneustes fossilis*) at BAU, sahar fish (*Tor putitora*) at IAAS, and climbing perch (*Anabas testudineus*) at CTU.

*Stocking Density:* For open ponds, 1 fish per m<sup>2</sup>; stocking size: 8 to 10 g. For cages, density will vary with treatments; stocking size: 8 to 10 g.

*Nutrient Inputs:* Caged fish will be fed twice daily with locally available homemade feed or commercial pelleted feed at rates of 5, 3 and 2% body weight per day during the first, second, and remaining months; no feed or fertilizers will be added into open ponds.

*Water Management:* Maintain at least 1.2 m depth.

*Sampling Schedule:*

Water quality: Standard CRSP protocol, biweekly water quality sampling, and monthly diel analysis at various depths.

Fish growth: Biweekly sampling for caged fish, monthly sampling for open-pond fish, and total harvests.

Soil and fish will be sampled at the beginning and end of the trial, while feed and/or ingredients will be sampled monthly for analyses of moisture, TN, and TP.

Nutrient budgets will be determined.

Partial enterprise budgets will be estimated to assess costs and value of fish crops.

*Statistical Design, Null Hypotheses, and Statistical Analysis:*

The trial will be conducted in a completely randomized design. For stinging catfish and climbing perch, there will be four stocking densities of caged fish (or four ratios of caged fish to open-pond

fish) as treatments in triplicates: 50, 100, 150, and 200 fish per m<sup>3</sup> in cages, giving the ratio of caged fish to open-pond fish as 1:1, 2:1, 3:1, and 4:1. There will also be a control in triplicates, in which ponds will be fertilized using local fertilization regimes and no cages will be placed in ponds. For sahar fish, the stocking densities in cages will be changed to 5, 25, 50, and 100 fish per m<sup>3</sup>.

Null hypotheses: There is no difference in growth and production of both caged fish and open-pond fish among tested stocking densities of caged fish. There are no effects on different stocking densities of caged fish on water quality, recovery efficiency of waste nutrients, and economic return.

The results of fish growth, nutrient budget, and water quality will be analyzed for significant differences among treatments using ANOVA and regression.

### **Regional Integration**

Pond culture is the most common aquaculture practice in the world. This integrated cage-cum-pond culture system can be adopted worldwide and may provide an appropriate option for small-scale rural farmers to make higher profits and diverse products.

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## Mitigating Environmental Impact of Cage Culture through Integrated Cage-Cum-Cove Culture System in Tri An Reservoir of Vietnam

Production System Design and Integration 4 (11PSDR4)/Study/Vietnam

### Investigators

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James S. Diana	US Principal Investigator	The University of Michigan
C. Kwei Lin	US Principal Investigator	The University of Michigan

### Objective

- 1) Assess the feasibility of the integrated cage-cum-cove system.
- 2) Assess effects of cages on water quality and plankton abundance in both coves and main reservoir.
- 3) Assess effects of cages on the fish production in coves.
- 4) Assess effects of accumulated cage wastes in bottom mud on terrestrial vegetation.

### Significance

Cage culture has long been practiced in rivers, lakes, and reservoirs in southeast Asia (Ling, 1977). In many cases, caged fish are fed with high protein diets; wastes derived from intensive cage culture cause pollution to the surrounding environment due to dissolved nutrients, uneaten feed, and metabolic by-products (Beveridge, 1984; Ackefors, 1986; Lin et al., 1990). Cage culture has been developed rapidly in Tri An Reservoir of Vietnam since the 1990s, with snakehead (*Channa striata*) as the major fish species for cage culture using trash fish as the main diets (Luu, 1998). Due to serious diseases of snakehead, the major cage culture species has recently changed to red tilapia (*Oreochromis spp.*). Environmental impacts of cage culture in reservoirs have become a major concern because wastes from cage culture is one of the major sources of pollution in Tri An Reservoir (Luu, 1998).

Another type of reservoir aquaculture is cove culture, in which fish are stocked and cultured in isolated bays of the reservoir using dams, barrier nets, or bamboo screens (Pillay, 1990; Li and Xu, 1995; Beveridge, 1996). The ideal cove for aquaculture is one that contains mainly a littoral zone of 1 to 2 m depth with alternating flood and exposure during rainy and dry seasons. Coves in Tri An Reservoir are usually cultured using net fences. Thus, most cove culture in Tri An Reservoir is practiced in extensive systems without fertilization and feeding, and the growth of stocked herbivorous and detritivorous species such as Chinese carps and Indian major carps is dependent on the natural foods derived from terrestrial vegetation in the inundated area, resulting in very low fish production.

An integrated cage-cum-pond system has been developed by Lin et al. (1990) and practiced for catfish-tilapia (Lin et al. 1990; Lin and Diana, 1995) and for tilapia-tilapia (McGinty, 1991; Yi et al., 1996; Yi and Lin, 2001). This integrated system reuses wastes derived from caged fish as a valuable resource to generate natural food for culture of filter-feeding species. Similar concepts can be introduced to reservoirs to develop an integrated cage-cum-cove system in Tri An Reservoir. In this integrated cage-cum-cove system, uneaten feed from cages can be reused by the fish in the open water of coves, dissolved nutrients and metabolic products from cages can stimulate the natural food production in coves, accumulated wastes on cove bottoms can serve as fertilizers for enhancing the growth of terrestrial vegetation after water withdrawal during the dry season, and terrestrial vegetation can enhance natural food production after inundation during the wet season. Therefore, the integrated cage-cum-cove system can minimize environmental pollution caused by cage culture in reservoirs.

The purposes of this study are to assess the feasibility of integrated cage-cum-cove systems, to assess the effects of cages on water quality and plankton abundance in both the cove and main reservoir,

## ELEVENTH WORK PLAN

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to assess the effects of cages on fish production in coves, and to assess the effects of accumulated wastes on the cove bottom on terrestrial vegetation.

### **Anticipated Benefits**

The results of this study could be used to enhance fish production with greater economic return from cove culture. The study would also provide an environmentally friendly strategy for the sustainable development of reservoir aquaculture. This integrated system will benefit the fish farmers throughout the region and other parts of the world.

### **Research Design**

*Location:* Truong Dang Aquaculture Cove, Tri An Reservoir, Vietnam. Lab work at Aquaculture Laboratory, University of Agriculture and Forestry, Vietnam.

### **Methods—Cove Research**

*Cage Facility:* Five cages of 32 m<sup>3</sup> size.

*Culture Period:* 180 days.

*Test Species:* Red tilapia (*Oreochromis* spp.).

*Stocking Density:* Cage—red tilapia at 50, 75, 100, 125, 150 per m<sup>2</sup>; cove—farmers' practice.

*Nutrient Input:* Cages—locally available feed; cove—none.

*Water Management:* Water level will be maintained at 1 m in cages.

#### *Sampling Schedules*

*Water Quality:* Monthly measurements of water quality at three depths at sites different distances away from cages.

*Plankton:* Monthly determination of plankton abundance at sites different distances away from cages.

*Moisture, TN, and TP in Fish Carcass:* Analyzed at stocking and harvest.

*Moisture, TN, and TP in Feed:* Analyzed for every batch of feed.

*Moisture, Bulk Density, TN, TP, and Organic C in Sediments:* At four cage sites and four other randomly selected sites at the beginning and end of one cycle and the beginning of the next cycle.

*Biomass, Moisture, Organic C, TN, and TP of Terrestrial Vegetation:* At four cage sites and four other randomly selected sites at the beginning of one cycle and the next cycle.

*Fish Growth:* Monthly sampling of red tilapia. No sampling for fish in the cove.

*Statistical Design, Null Hypothesis, and Statistical Analysis:* Changes of water quality and plankton abundance over time and different distances away from the cages up to 100 m from the cove in the main reservoir.

Difference in biomass of terrestrial vegetation at the beginning of the cycle and next cycle.

Difference in nutrients in the cove sediments at four cage sites and other four sites selected randomly.

Fish production in the cove will be compared to that in the previous year and that from other similar coves. Fish production in cages in the cove will be compared to that in cages in open water.



The null hypothesis is that cages have no effects on water quality, plankton abundance, soil nutrients, terrestrial vegetation, and fish production, and nutrient recovery.

The data will be analyzed using ANOVA.

## **Regional Integration**

In many Asian countries, reservoirs have been developed for fish production, making a significant contribution to overall inland fish production. However, the production is usually low and faces increasing fishing pressure. Development of the integrated cage-cum-cove system will enhance fish production and minimize environmental pollution from cage culture in reservoirs in the region and other parts of the world.

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### Optimization of Nitrogen Fertilization Regime in Fertilized Nile Tilapia Ponds With Supplemental Feed

Production System Design and Integration 5 (11PSDR5)/Experiment/Thailand

#### Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology, Pathumthani, Thailand
James S. Diana	US Principal Investigator	The University of Michigan
C. Kwei Lin	US Co-Principal Investigator	The University of Michigan

#### Objectives

- 1) Optimize nitrogen input in Nile tilapia ponds with supplemental feed.
- 2) Assess effects of different fertilization regimes on fish production.
- 3) Assess effects of different fertilization regimes on water quality and pond effluent.

#### Significance

Nile tilapia (*Oreochromis niloticus*) is commonly produced in semi-intensive culture in southeast Asia using fertilization to increase primary production (Boyd, 1976; Diana et al., 1991). There is voluminous literature available for optimization of fertilization rate in fish ponds applied with inorganic or organic fertilizers or their combinations as the sole nutrient inputs (e.g., Hickling, 1962; Boyd, 1976, 1978; Olah, 1986; Green et al., 1990; Diana et al., 1991; Knud-Hansen et al., 1991; Edwards et al., 1994; Lin et al., 1997). Also, research has been done with different combinations of fertilizers and feeds (e.g., Diana et al., 1994; Milstein et al., 1995). However, almost all research aiming to optimize supplemental feeding rate has been conducted in ponds with fixed fertilization rates. Few experiments have been done on optimizing fertilization regimes in fertilized ponds with supplemental feeds.

Diana et al. (1994) determined that the feeding rate of 50% ad lib was optimal in ponds fertilized at a fixed rate of 28 kg N and 7 kg P ha<sup>-1</sup> wk<sup>-1</sup>, and Diana et al. (1996) also determined that the initial addition of supplemental feed at 50% ad lib once fish reached 100 g is the most cost-effective way to produce large tilapia. While this fertilization rate is recommended when fertilizer serves as the sole nutrient input for Nile tilapia culture in the tropics (Knud-Hansen et al., 1991), the nutrients may become excessive in ponds with supplemental feeding as substantial amounts of nutrients are also released from feeding wastes to pond water for phytoplankton production (Lin, 1990; Lin and Diana, 1995; Yi et al., 1996; Yi and Lin, 2001; Yi et al., 2001). The natural foods in fertilized ponds increase efficiency of supplemental feeds significantly as indicated by lower food conversion ratios (Diana et al., 1994). Thus, it is ecologically and economically important to maintain adequate production of natural foods in fed ponds with balanced nutrient inputs from both external fertilization and internal wastes. The rate of external fertilization should be adjusted according to amount of nutrients derived from feeding. This will result in more efficient utilization of nutrients, better water quality, lower production cost, and reduced nutrient load in pond effluents. The proposed study is a follow-up of earlier PD/A CRSP research and will optimize nitrogen addition to combine with feeding for Nile tilapia culture.

#### Anticipated Benefits

Results of this study will be used to develop an appropriate nitrogen fertilization strategy for Nile tilapia production in ponds with supplemental feed. It will benefit fish producers in Asia and other countries when extended on a large scale. The study itself will demonstrate biological and economic efficiency to be gained by adjustments in nitrogen fertilization rates.

#### Research Design

Location: AIT.

## *Methods–Pond Research*

*Pond Facility:* 15 earthen ponds of 200 m<sup>2</sup> in surface area.

*Culture Period:* 180 days.

*Test Species:* Nile tilapia (*Oreochromis niloticus*).

*Stocking Density:* 3 fish per m<sup>2</sup>.

*Nutrient Input:* Ponds will be fertilized using urea and TSP at a fixed rate of 28 kg N and 7 kg P ha<sup>-1</sup> wk<sup>-1</sup> in all ponds until tilapia reach 100 g. Then tilapia will be fed at a feeding rate of 50% ad libitum with floating pelleted feed (30% crude protein) in all ponds, and nitrogen input will be adjusted in various treatments, while phosphorus rate will be kept unchanged.

*Water Management:* Maintain at 1 m depth.

## *Sampling Schedule*

*Water Quality:* Standard CRSP protocol, biweekly water quality sampling, and monthly diel analysis at various depths.

*Soil Samples:* Taken and analyzed for moisture, total N, and total P at the beginning and end of the experiment.

*Fish Growth:* Monthly sampling and total harvests. Partial economic budgets will be assessed.

Nutrient budget will be determined. Nutrients contained in pond effluent will be determined by taking water samples at different times from pump outlets during water discharge at harvest.

## *Statistical Design, Null Hypothesis, and Statistical Analysis:*

The experiment is a completely randomized design with five nitrogen rates as treatments with three replicates each: 0, 25, 50, 75, and 100% of 28 kg N ha<sup>-1</sup> wk<sup>-1</sup>; that is, 0, 7, 14, 21, and 28 kg N ha<sup>-1</sup> wk<sup>-1</sup>.

The null hypothesis is that nitrogen input rates have no effect on the growth of Nile tilapia, nutrient utilization efficiency, economic return, water quality, or pond effluent quality.

The results of fish growth and water quality will be analyzed for significant differences among treatments using ANOVA and regression. Also, feed conversion efficiencies and nutrient utilization efficiency will be evaluated over the entire culture period for differences among treatments.

## **Regional integration**

In many Asian countries, the addition of supplemental feeds into fertilized ponds is becoming more and more popular for tilapia production. This study will provide optimal fertilization regimes for tilapia culturists to allow them to achieve higher economic returns and to reduce nutrient content in pond effluents compared to current culture systems. The results from this study will be used by AIT outreach and country extension agents to advise farmers of better culture practices.

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## Workshop on Fertilization Strategies for Pond Culture in Bangladesh

Production System Design and Integration 6 (11PSDR6)/Activity/Bangladesh

### Investigators

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Md. Abdul Wahab	HC Principal Investigator	Bangladesh Agricultural University, Mymensingh, Bangladesh
James S. Diana	US Principal Investigator	The University of Michigan
C. Kwei Lin	US Principal Investigator	The University of Michigan

### Objectives

- 1) Disseminate information of PD/A CRSP fertilization technologies.
- 2) Transfer appropriate fertilization strategies developed by the PD/A CRSP to aquaculture extension agencies in Bangladesh.
- 3) Enhance cooperation between the PD/A CRSP with academic institutions, government agencies, nongovernmental organizations (NGOs), and international organizations in Bangladesh.

### Significance

Bangladesh is one of the most densely populated countries in the world. Fisheries and aquaculture in particular are vital to Bangladesh's national economy in terms of nutrition, income, employment generation, and foreign exchange earning (Alam et al., 1996). Currently, approximately 80% of the animal protein supply for residents is provided by fish, but population growth is rapidly overwhelming the productive potential of the Bangladesh fishery (O'Riordan, 1992). Since the 1960s, per capita availability of fish has dropped from 12 kg to only 7 kg; moreover, among lower income groups, per capita consumption is only 4.4 kg. For the poorest of the poor, fish is simply unaffordable (O'Riordan, 1992). Thus, aquaculture plays more and more important roles to meet the nutritional needs of Bangladesh people.

Bangladesh has a variety of aquaculture and fisheries projects that have been funded by international aid. Many nongovernmental organizations (NGOs) such as PROSHIKA, BRAC, and CARITAS have been promoting aquaculture development independently through their own extension networks in Bangladesh.

Aquaculture is commonly practiced using polyculture of four to seven species of Indian and Chinese carps in manured and/or fertilized ponds (Wahab et al., 1991). In spite of extensive research that has been conducted on fertilization in carp polyculture ponds in many parts of the world, such information in Bangladesh is rather scanty (Haq et al., 1993). Fish production is quite low in Bangladesh, averaging 2,800 kg ha<sup>-1</sup> yr<sup>-1</sup> (Department of Fisheries, 1999). In rural aquaculture ponds, fish production is often lower than 1,500 kg ha<sup>-1</sup> yr<sup>-1</sup>. NGOs have been working with farmers to increase fish production. However, different NGOs recommend different fertilization regimes to farmers, and these regimes do not all seem to increase yields. Fertilization regimes should vary with different local conditions such as soil and source water. In some cases, the same farmers receive very different recommendations on fertilization regimes from different extension partners. Both over- and under-fertilization may cause adverse effects on fish production, water quality, pond effluents, and economic returns. It is important to recommend appropriate fertilization strategies to farmers in order to maximize fish production, maintain good water quality, reduce environmental impact, and maximize economic returns.

### Anticipated Benefits

This workshop will enhance knowledge and capabilities of the institutions involved in aquaculture/fisheries outreach in fertilization strategies for pond culture in Bangladesh and increase impacts of the

## ELEVENTH WORK PLAN

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PD/A CRSP to Bangladesh. Bangladesh researchers, extension staff of government agencies and NGOs, and fish farmers will benefit from the experiences, research results, technologies, and approaches of the PD/A CRSP through this workshop.

### Activity Plan

This workshop will be held for one day at the BRAC Center in Dhaka, Bangladesh, and will have about 30 to 50 participants from academic institutions, government agencies, NGOs, and international organizations in Bangladesh. The following topics will be presented in the workshop:

- 1) Brief introduction to PD/A CRSP activities in the past two decades.
- 2) Pond aquaculture in Bangladesh—a status review.
- 3) Pond fertilization practices in Bangladesh.
- 4) On-station trials of different fertilization regimes used in Bangladesh conducted by the PD/A CRSP.
- 5) On-farm trials of different fertilization regimes used in Bangladesh conducted by the PD/A CRSP.
- 6) Fertilization strategies for tilapia culture developed by the PD/A CRSP.
- 7) Environmental impacts of the intensification of pond culture.

Workshop proceedings will be published and distributed to extension agencies in Bangladesh and other countries in this region.

### Regional Integration

Polyculture of Indian major carps and Chinese carps is commonly practiced in south Asia, and also tilapia culture will be expanded soon. Improvements in fertilization regimes will provide an appropriate pond culture strategy for small-scale rural farmers in terms of technical, environmental, and economic aspects. It will be of interest to all NGOs involved in aquaculture outreach.

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## Broodstock Development and Larval Feeding of Amazonian Fishes

Indigenous Species Development 1 (11ISDR1)/Experiment/Peru

### Investigators

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Fred W. Chu	Graduate Student	Southern Illinois University at Carbondale
Maria Esther Palacios	Graduate Student	The Ohio State University

### Objective

- 1) Improve the quality of progenies by using different live and formulated diets for larval paiche (*Arapaima gigas*) and *Pseudoplatystoma* sp.

### Significance

*Arapaima gigas*, commonly known as paiche (Spanish) or pirarucu (Portuguese), is the largest freshwater fish of the Amazon in the world (measuring up to 10 feet long and weighing up to 200 kg) has been listed in the 2000 IUCN Red List of Threatened Species since 1996 (Hilton-Taylor, 2000). It could be found only in the Amazon and Essequibo River basins surrounding Peru, Guyana, and Brazil. In the last 30 years, illegal fishing and poaching have drastically reduced the populations of *Arapaima* in their local habitats. Studies on artificial propagation of this fish are important to alleviate the condition of poverty-stricken rural areas by providing food and source of income for the Amazon people (Gram et al., 2001). Being a top-of-the-food-web species, maintaining paiche populations in the wild will also mean ecological balance in the Amazon rainforest.

In 2001, the first batch of paiche matured in IIAP (Pucallpa and Iquitos), Peru and observations on spawning behavior (nesting), duration and frequency of breeding, and fecundity were made (Mariano and Alcántara, personal communication). Part of the progenies were removed after a few days (as early as six to seven days old) from parental care and raised in captivity (hatchery tanks). Development of larval feeds for raising *Arapaima* in captivity will resolve the problem of providing natural food for these fishes and developing domesticated stock that relies on artificial diets. Interestingly, information

on natural spawning in ponds was made available in Brazil during the same year (Imbiriba, 2001).

Considering that fish is a major part of the diet of Amazon communities (Brazil and Peru; Eckmann, 1983), aquaculture production of this species will maintain their consumption without overfishing natural populations and, in effect, promote the utilization and conservation of wild stocks in the Amazon rainforest. Controlled reproduction of paiche may also considerably limit illegal international trade of this and other osteoglossid species (Matsumura and Millikin, 1984). There is increasing pressure in the conversion of the flood plains to rice paddies and cattle pastures (deforestation; Achard et al., 2002) and viable aquaculture may prevent this trend.

*Pseudoplatystoma fasciatum*, *P. tigrinum*, and *P. corruscans* are considered as potential aquaculture species in South America (Kossowski, 1996). These fish have excellent quality, and taste of the meat and low amount of bones (Martino et al., 2002) make it one of the most appreciated fish in the region. Wild populations have decreased considerably due to overexploitation of stocks along the Amazon (Cerdeira et al., 2000). In Peru, spawning occurs from February to March and some fish farmers have considerable success in domesticating these fish (Alcántara, personal communication). In Brazil, good growth rates were observed in *P. corruscans* in captivity (Fujimoto and Carneiro, 2001). Our proposed study could boost the interests in promoting the aquaculture of these indigenous species by providing information on its nutritional requirements.

### **Quantified Anticipated Benefits**

The proposed study aims to investigate key aspects of nutrition and reproduction biology of *Arapaima* sp. and *Pseudoplatystoma* sp. in order to improve or develop sustainable aquaculture technology for these species.

Through our collaborative effort with Peruvian investigators we will be able (1) to improve quality of larvae obtained from *Arapaima* and *Pseudoplatystoma* broodstock under culture conditions and (2) to develop the procedures of first-feeding of *Arapaima* and *Pseudoplatystoma*. The main beneficiaries of this research will be the local producers in the Peruvian Amazon and neighboring countries (Brazil, Colombia, Ecuador, Bolivia). Development of the technology of intensive growth of these species and stocking 4 to 6 week old juveniles will dramatically increase their survival and efficiency of production.

The Amazonian catfishes could be cultured in monoculture or polyculture systems in order to control the native cichlids like *Cichlassoma* and *Aequidens*. All species of catfishes reach large sizes (20 to 40 kg) and have a wide distribution throughout the Amazonian basin in South America (Colombia, Venezuela, Brazil). The experiences gained with these species will be applicable in many countries of the region. *P. tigrinum* is an attractive species for the aquarium business; therefore, development of aquaculture technology will reduce pressure on natural stocks and create an additional source of income for local fish farmers.

More importantly, this study will also contribute towards institutional strengthening by providing training for IIAP staff and a graduate student from Peru on various aspects of fish nutrition and reproduction.

### **Research Design**

**Location of Work:** Some experiments in Peru will be conducted in IIAP-Iquitos or Pucallpa, two regional research centers in Loreto Province. IIAP is a non-profit research institution that is charged with alleviating the socioeconomic conditions of the Amazonian farmers by conducting research on sustainable development and protection of natural resources.

### **Methods**

**Objective 1:** Improve progeny through the usage of live and formulated larval diets for *Arapaima* sp. and *Pseudoplatystoma* sp.

This objective will be carried out simultaneously in IIAP-Iquitos and Pucallpa. Larvae of the species available will be obtained from fish induced to spawn by hormonal injections (*Pseudoplatystoma*

only) or collected in the pond following observation of spawning behavior. The feeding experiment will be conducted in a flow-through system consisting of 12 cylindrical, flat bottom tanks (3 tanks per dietary treatment) supplied with aeration. Water quality will be monitored throughout the larval rearing process. Temperature (26 to 28°C) and dissolved oxygen (5 to 6 mg L<sup>-1</sup>) will be monitored on a daily basis with weekly measurements of total ammonia-nitrogen and pH. Two days after yolk resorption, catfish larvae will be randomly distributed at a density of 200 larvae per tank and fed at a restricted ration up to 90% satiation for 2 to 4 weeks. An improved food dispenser will be installed (Charlon and Bergot, 1986) for the formulated diets. At the beginning of the experiment, samples of 10 larvae will be weighed. Larvae will be fed six diets: (1) freshly hatched nauplii, (2) decapsulated cysts, and (3) local plankton produced in ponds. Three experimental diets will be prepared at OhSU. These diets are based on casein-gelatin and casein-hydrolysates (Palacios et al., in preparation). Larval samples (n = 20) will be taken every week from each tank and fixed in buffered formalin for biometric measurements. At the end of the experiment, growth performance will be evaluated in terms of final individual body weight, survival (%), specific growth rate (SGR, %), and weight gain (%). Fish from each dietary treatment also will be sampled for proximate body analysis (water, protein, lipid, ash).

*Statistical Analysis:* Analyses will be performed using the Statistical Package for the Social Sciences Version 10.1 (SPSS 10.1). Data on growth performance and survival will be subjected to one-way analysis of variance (ANOVA) followed by a comparison of means using the Least Significant Difference (LSD) Test (Steel and Torrie, 1980). Normality and homogeneity of variance tests will be performed on raw data. Sample distributions violating assumptions will be log-transformed before analysis. Data, expressed as percentages, will be arc sine-transformed before analysis. All differences will be regarded as significant at P < 0.05.

### **Regional Integration**

Aside from Peru, *Arapaima* sp. and *Pseudoplatystoma* sp. are economically important in other countries in South America, especially in Brazil, Argentina, Bolivia, and Guyana. It is then evident that studies that will promote the artificial propagation of these fishes will improve the profitability of aquaculture operations of fish farmers and consequently economic conditions of rural communities in these countries. In addition, readily available techniques of farming these fishes will reduce the pressure of catching fish from the wild. The studies we proposed are consistent with the role of IIAP as an international center in the upper Amazon. Results from these studies will be published as fact sheets and distributed to key academic and research institutions in the region to promote interests among researchers working with these fishes.

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### Controlled Reproduction of an Important Indigenous Species, *Spinibarbus denticulatus*, in Southeast Asia

Indigenous Species Development 2 (11ISDR2)/Study/Vietnam

#### Investigator

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#### Objectives

- 1) Understand the seasonal pattern of gonadal development, sexual maturation, and various reproductive parameters.
- 2) Induce this species to spawn in a controlled environment using both natural and artificial methods.

#### Significance

Chinese and Indian major carps and tilapias make up over 90% of freshwater species cultured and over 95% of this production comes from Asia (FAO, 2000). All of these species are not native to southeast Asia (Liste and Chevey, 1932). Although culture of introduced species is profitable, they have also been implicated in either displacement of indigenous species or introgression with local species (Ogutu and Hecky, 1991; De Jongh and Van Zon, 1993; Goel, 2000). Consequently, local indigenous species composition is negatively impacted. Additionally, exotic species are susceptible to diseases. For example, grass carp is prone to local environmental stressors such as the red spot disease (presumably a viral disease, Supranee Chinabut, personal communication). Grass carp, a primary cultured species for the rural poor, has been severely affected by this pathogen to a degree where many poor farmers of north Vietnam have abandoned culture of this fish, a primary animal protein source in their diet. There is a need to identify an alternative species to grass carp, preferably from within the pool of indigenous species.

Southeast Asia is known to possess one of the largest diversities of freshwater fish species in the world (FAO, 1993). Unfortunately, comparatively few species from this region have been brought under cultivation partly due to lack of sufficient knowledge on reproduction and seed production. *Spinibarbus denticulatus* is an example of such a species that has significant potential for aquaculture, particularly for a low-input system of north Vietnam (Red River Delta System). This herbivorous species has a diet consisting of plankton and macrophytes—very similar to that of the grass carp (Bao, 1989). One of the most attractive features of this species is that it is resistant to red spot disease, even when grown together in the same cage with infected grass carp. It is also a fast growing species and is cultured primarily by stocking in ponds and cages with seed collected from the wild. Culture of this species is constrained by a limited supply of seed in the wild. We know of no publication that characterizes reproduction of this species beyond some basic biology. Preliminary studies at the Research Institute for Aquaculture No. 1 in Hanoi indicate that this species could be spawned in captivity and could respond to natural and hormonal stimuli for spawning. A more thorough study is needed to better understand the reproductive biology and to produce seed using low-cost and relatively simple hatchery techniques.

#### Anticipated Benefits

Successful mass production of *S. denticulatus* seed would:

- 1) Lower the seed collection pressure in natural populations.
- 2) Increase the availability of seed in a more predictable manner at a lower cost with potential for making this fish more widely cultured throughout southeast Asia.
- 3) Replace exotic species such as grass carp.



- 4) Add one more low-cost freshwater fish to the list of aquaculture species with the potential to directly benefit the rural poor.

## **Research Design**

*Location:* Research Institute for Aquaculture No. 1, Vietnam. This is the lead aquaculture institute in Vietnam with over 12 trained scientists on location. It has functioning wet and dry lab and a number of well-managed ponds on campus. Additionally, it has several satellite campuses where a majority of this study will be conducted.

*Methods:* The initial experiment will provide us with the basic knowledge on hormonal profile, sexual maturation, and gonadal development relative to the breeding season. It will also evaluate responses to environmental and exogenous hormones.

One hundred pairs of adult fish will be held in two 400 m<sup>2</sup> earthen ponds. All experimental fish will be marked using pit-tags. Blood samples will be collected from both males and females once every two weeks for hormonal profile. Gonad maturation and spawning readiness will be assessed (during the early to late spawning season—believed to be spring) by monitoring vitellogenic stages, egg diameter, GVBD, and nuclear migration as well as relative gonad weight (GSI).

### *Response to Environmental and Hormonal Stimuli*

Environmental parameters tested:

- 1) Increase water temperature by lowering of the water depth.
- 2) Simulate rainfall by increasing the water level and placing a sprinkler system on the 200 m<sup>2</sup> pond surface.

Response to hormonal stimulation:

Two hormones (carp pituitary extract or GnRH + domperidone, commonly available in Vietnam) will be tested using slight variation on standard dose used for Chinese carp. The final oocyte maturation and spawning will be monitored.

Further optimization of a specific hormone will be carried out based on the findings above.

### *Larval Rearing*

Newly hatched larvae will be placed in clear and green water systems in the hatchery. Both will be fed with live rotifers five times per day until they are ready to feed on fine particles of artificial feed. Growth rate, feeding vigor, and survival will be monitored for four weeks post hatch.

### *Experimental Design, Null Hypothesis, and Statistical Methods:*

**Experiment 1:** Sexual maturation. Null hypothesis: There is no seasonal effect on sexual maturation of this species. Sampling will be done every two weeks and increase sampling frequency as the spawning season peaks using 10 males and 20 females.

**Experiment 2:** Environmental control of maturation and spawning. Null hypothesis: Temperature and rain have no impact on advanced maturation and spawning. Twenty pairs will be placed in triplicate ponds. Two separate ponds containing 20 pairs each will not receive treatment and will function as the control.

**Experiment 3:** Forty females will receive three doses (high, medium, low) of each hormones (carp pituitary and GnRH) (five fish per treatment). Controls will receive saline injection.

Tables and figures will summarize the information from Experiment 1. Mean significant differences will be tested using ANOVA in Experiments 2 and 3.

## **Regional Integration**

This is a cyprinid species similar to grass carp in its feeding habits, growth, and flesh texture (except it has no intramuscular bones). It would potentially replace grass carp where there have been difficulties with red spot disease. However, in the regions where grass carp is commonly cultured and not affected

by disease, *S. denticulatus* would be complementary as they can be stocked together and fed the same diet. While commercial potential is unexplored, it has significant implications for resource poor farmers and rural communities in south and southeast Asia because of low input required for culture of this species.

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**Elimination of Methyltestosterone from Intensive Masculinization Systems:  
Use of Ultraviolet Irradiation of Water**

Water Quality and Availability (11WQAR1)/Experiment/Mexico

**Investigators**

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**Objectives**

- 1) Determine if the 17a-methyltestosterone that escapes to the water after dietary treatment of tilapia fry can be eliminated from intensive masculinization systems.
- 2) Evaluate the efficacy of a new technology for clean effluents in aquaculture.

**Significance**

All-male populations are used in tilapia (*Oreochromis* spp.) aquaculture because the culture of mixed-sex populations often results in precocious maturation and early reproduction (Schreck, 1974; Mires, 1995). Furthermore, all-male tilapia populations are desirable because males achieve a larger final size than females (MacIntosh and Little, 1995).

Masculinization of tilapia fry by oral administration of 17a-methyltestosterone (MT) is considered the most successful method employed; however, under certain conditions this technique is sometimes less favorable. Furthermore, significant “leakage” of MT into the pond environment may occur from uneaten or unmetabolized food. This leakage poses a risk of unintended exposure of hatchery workers, as well as fish or other non-target aquatic organisms, to the steroid or its metabolites.

In recent studies (Contreras-Sánchez, 2001), we found that masculinization of fry through dietary treatment with MT resulted in the accumulation of MT in sediments which, produced both intersex fish and females with altered ovarian development. In systems where substrate was not present, there were higher concentrations of MT in the water and lower (sometimes null) masculinization rates than in systems with either soil or gravel. We found that charcoal filtration of water from systems where substrate was not present lowered the amount of MT in water to almost background levels and the treatment resulted in almost complete masculinization of all three broods tested (100, 98, and 100% males, respectively). Apparently, the recommended dose of MT for masculinizing tilapia is higher than needed and a significant portion of it separates from the food and remains either in suspension in the water for the short term or persists in the sediments over the long term (Contreras-Sánchez, 2001). In the cited study, we recommended the use of activated charcoal filtration systems to eliminate excess MT to increase masculinization and to prevent potential risks to humans of unintended exposure to MT due to contamination of water and soils in farms. Alternative techniques, such as ultraviolet (UV) irradiation of water, may provide a more efficient method for removal of MT and eliminate the handling of MT-laden charcoal.

In Mexico, the use of MT for masculinizing tilapia fry is a new activity. Little is known regarding the use of MT and the scarce information available to hatchery producers and fish farmers does not deal with the potential risks of this practice. In the southeastern region of the country, hatchery production goals have not been reached and the methods used are far from being efficient. Despite almost 30 years of tilapia farming in Mexico, the use of mixed-sex populations is still a very common practice and as a result, the productivity of many hatcheries and farms is severely affected.

## ELEVENTH WORK PLAN

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Methyltestosterone is a light sensitive hormone which is subject to photodegradation (Budavari et al., 1989; Sigma Chemical Company, 1994). It is well known by aquaculturists that charcoal used to purify water can be reactivated by sunlight exposure. The type of light most likely responsible for photodegradation is UV-B (wavelengths of 280 to 315 nm). Methyltestosterone absorbs UV light strongly at a wavelength of 254 nm, which is in the UV-C part of the spectrum (100 to 280 nm), and absorbs UV weakly in the UV-B area of the spectrum. Unlike UV-B, UV-C is quickly absorbed in the atmosphere and does not reach the earth's surface. Since MT does not absorb UV-B very effectively, treatment with irradiation at 254 nm should be much more effective than exposure to sunlight or UV-B. Virtually nothing is known about the amount of exposure to UV needed to remove MT nor of possible metabolites produced during photodegradation. Commercial ultraviolet water sterilizers are currently being used by some growers in Central America to destroy pathogens. These sterilizers emit UV light at a wavelength of 254 nm.

We propose the use of intensive systems for masculinizing tilapia fry using MT-impregnated food at a large scale where excess MT is eliminated from the water by means of continuous filtration through UV sterilizers. Removal of MT should both increase masculinization rates and reduce the amount entering substrates which could affect other aquatic organisms. This method may allow for the production of large numbers of all-male populations of tilapia fry using a reliable technique compatible with the proposed Best Management Practices (BMPs) for aquacultural systems. Ultraviolet sterilizers are relatively cheap, available in many sizes for different volumes of water in aquaculture systems, and can be readily obtained in southern Mexico.

If successful, this method can be transferred to tilapia hatcheries that play an important role in poor areas of the states of Tabasco and Chiapas, Mexico. The use of reliable and efficient masculinizing methods in the hatcheries will benefit thousands of small-scale fish farmers who currently see their productivity negatively affected by the use of mixed-sex populations of tilapia. A series of training workshops will be developed and offered to different audiences in the communities of Tabasco and Chiapas to ensure that this methodology is effectively transferred to its final users. Technical workshops will target hatchery managers, extension agents and university students (many of whom will become workshop instructors over time). Public extension workshops will be tailored to the cultural characteristics of the target audience and will be offered to fish farmers, farm workers, and selected community leaders.

### **Anticipated Benefits**

The development of clean technologies for aquacultural practices will positively impact the production of sex-reversed tilapia fry.

The use of UV sterilizers in intensive systems for masculinizing tilapia will improve safety in handling masculinizing steroids, produce clean effluents, and potentially increase efficiency of masculinization.

### **Research Design**

#### **Experiment 1: Removal of MT from water by solar or UV irradiation (non-pond research)**

*Site:* Experiments will be conducted at Oregon State University.

*Methods:* Expose water containing MT to either direct sunlight or UV light.

*Laboratory Facility:* Oregon State University's Fish Performance and Genetics Laboratory at Smith Farm, Corvallis, eight aquaria, flow through system for UV exposure, MT radioimmunoassay (RIA) for quantifying MT concentrations.

*Water Management:* Water temperature will be maintained at 28 to 30°C for UV irradiation and at ambient temperature for sunlight exposure.

*Sampling Schedule:* The experiment consists of five treatments done in duplicate; all exposures will be

for 48 hours; all water samples will be collected at 2, 4, 8, 24, and 48 hours:

- control water exposed to sunlight.
- control water exposed to UV light.
- MT treated water exposed to sunlight.
- MT treated water exposed to UV light.
- MT treated water not exposed to any light.

Untreated control water in aquaria (50 liter) will be placed in direct sunlight. UV transparent glass will be placed on top of the aquaria to allow for exposure to UV light. Water samples (12 ml) will be extracted with ether and MT content determined by RIA. Control water in 50 liter aquaria will be exposed to UV light at 254 nm in the dark by passing circulating water through a UV sterilizer at a comparable rate to that of large masculinization systems. Experimental water will be treated with MT at 5, 25 or 50 mg l<sup>-1</sup> and exposed to either sunlight or UV in the same way as for control water. These doses represent a range of MT (5 to 15 mg l<sup>-1</sup>) seen in previous experiments after fry have been fed MT for sex inversion at a dose of 60 mg kg<sup>-1</sup> feed for 28 days (Contreras-Sánchez, 2001). Positive controls will be generated by adding MT to water and circulating it through the system in the dark with the UV lamp turned off.

*Statistical Methods and Hypotheses:* H<sub>0</sub>1: MT is not detectable in control water at any time. H<sub>0</sub>2: MT is detectable in MT treated water at each exposure and at all sampling times in a dose-dependent manner and independently of type of light exposure. MT content of water will be compared between either control or treated water, UV and sunlight exposed, and for exposure time in treated water using ANOVA with  $p < 0.05$ . The total dose of UV irradiation required to remove the majority of MT from water will be calculated and applied to the following three field experiments.

### Experiment 2: Elimination of MT from the water of intensive sex-inversion systems

*Site:* Experiments will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, Mexico.

*Methods:* Oral administration of MT (dose = 60 mg kg<sup>-1</sup>) in concrete tanks (2 m<sup>2</sup>)

*Laboratory and Pond Facility:* Universidad Juárez Autónoma de Tabasco; 1 earthen pond (200 m<sup>2</sup>), 50 net cages (1 m<sup>3</sup>), 3 concrete tanks (8 m<sup>3</sup>), 8 concrete ponds (2 m<sup>2</sup>), 3 grow-out ponds (200 m<sup>2</sup>), a total of 200 females and 65 males for production of fry.

*Culture Period:* 3 months

*Stocking rate:* 2,500 fry per m<sup>2</sup>

*Test Species:* Nile tilapia (*Oreochromis niloticus*)

*Nutrient Inputs:* None

*Water Management:* Water will be recirculated through 2,000 liter tanks. The MT tanks will receive MT-treatment recirculated water; the control tanks will receive control recirculated water. A 25% water exchange will be performed twice a week.

*Sampling Schedule:* The experiment consists of four treatments, all treatments will be done in duplicate:

- fry fed control food for 28 days; water not recirculated through a UV sterilizer.
- fry fed control food for 28 days; water recirculated through a UV sterilizer.
- fry fed MT at 60 mg kg<sup>-1</sup> food for 28 days; water not recirculated through a UV sterilizer.
- fry fed MT at 60 mg kg<sup>-1</sup> food for 28 days; water recirculated through a UV sterilizer.

Water (12 ml) samples will be collected from each treatment group each day. Samples will be frozen (-20°C) and preserved until processing. All samples will be extracted using ether and the concentration of MT determined by RIA (at OSU). At the end of a three-month grow-out period, a sub-sample of the

## ELEVENTH WORK PLAN

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tilapia in each experimental unit (100) will be killed with an overdose of anesthetic (MS-222) to determine if the treatment with MT resulted in masculinization. The following water quality parameters will be measured weekly: pH, dissolved oxygen, and temperature.

*Statistical Methods and Hypotheses:* H<sub>0</sub>1: MT is not detectable in control water at any time. H<sub>0</sub>2: MT is detectable in water at any time during treatment of tilapia fry with MT-impregnated food independently of the use of UV sterilizers. MT content of water will be compared between either control or treated water, UV exposed and unexposed, and for exposure time in treated water using ANOVA with  $p < 0.05$ . H<sub>0</sub>3: Administration of MT-feed to tilapia held in systems with or without UV sterilizers produces fish with the same sex ratios as the controls. Sex ratios will be compared by a Chi-square test.

### Experiment 3: Determine biological activity of photodegraded MT metabolites

*Site:* Experiments will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, Mexico.

*Methods:* New groups of non-MT fed fish will be placed in tanks containing water from each of the groups from Experiment 2 and examined for sex inversion.

*Laboratory and Pond Facility:* Universidad Juárez Autónoma de Tabasco; 1 earthen pond (200 m<sup>2</sup>), 50 net cages (1 m<sup>3</sup>), 3 concrete tanks (8 m<sup>3</sup>), 8 concrete ponds (2 m<sup>2</sup>), 3 grow-out ponds (200 m<sup>2</sup>), a total of 200 females and 65 males for production of fry.

*Culture Period:* 3 months

*Stocking rate:* 2,500 fry per m<sup>2</sup>

*Test Species:* Nile tilapia (*Oreochromis niloticus*)

*Nutrient Inputs:* None

*Water Management:* Water will be recirculated through 2,000 liter tanks. A 25% water exchange will be performed twice a week.

*Sampling schedule:* Following removal of fish from treatments in Experiment 2, new groups of fish will be added and fed control (non-MT treated) food for 28 days. Water and fish will be sampled and analyzed the same as in Experiment 2.

*Statistical Methods and Hypothesis:* H<sub>0</sub>1: MT is not detectable in control water at any time. H<sub>0</sub>2: MT is detectable in water previously exposed to MT at any time and independently of the use of UV sterilizers. MT content of water will be compared between either control or treated water, previously exposed to UV and unexposed, and for time in previously treated water using ANOVA with  $p < 0.05$ . H<sub>0</sub>3: Tilapia held in systems previously exposed to MT, with or without UV sterilizers, have the same sex ratios as fish held in control water. Sex ratios will be compared by a Chi-square test.

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### Use of a Wetland to Treat Effluent from a Flow-Through Aquaculture System

Water Quality and Availability 2 (11WQAR2)/Experiment/Brazil

#### Investigators

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Claude E. Boyd	US Principal Investigator	Auburn University

#### Objectives

- 1) Compare water quality in fish ponds with and without water exchange.
- 2) Evaluate the effectiveness of a wetland to improve the quality of effluents from ponds with water exchange.
- 3) Use findings from the research to propose best management practices (BMPs) for pond effluents.

#### Significance

In Brazil, pond fish culture often is conducted in flow-through systems and effluent is discharged into natural waters. Also, when ponds are harvested effluent is discharged into natural waters. Productivity in ponds is increased with fertilizers and feeds, and waters may have high concentrations of nutrients, organic matter, and suspended solids (Silva and Anderson, 1995; Boyd et al., 2000). A study by McGee and Boyd (1983) suggested that modest rates of water exchange used in channel catfish production did not significantly improve water quality. There have been no studies of the benefits of water exchange in pond culture in Brazil, although continuous water exchange did improve water quality in culture tanks (Sipaúba-Tavares et al., 2000). Settling basins can be used to improve the quality of pond effluents (Boyd and Queiroz, 2001), but natural or created wetlands also have been suggested as sedimentation areas and biofilters for improving the quality of pond effluents (Schwartz and Boyd, 1995; Sipaúba-Tavares, 2000). However, more research is needed to determine the extent to which aquaculture effluents can be improved by wetland treatment. It is often suggested that wetland biofilters can purify aquaculture effluents enough to permit their reuse in aquaculture, but more research is needed to verify that wetlands can improve the quality of pond effluent so that it is equal in quality to the original source water.

This study can be significant to Brazilian aquaculture by determining if modest water exchange in culture ponds is useful in improving water quality. It also can ascertain the degree of improvement in water quality that can be expected from wetland treatment of pond effluents.

#### Anticipated Benefits

The main output of the research will be to demonstrate the degree of improvement in water quality that can be expected which modest water exchange is used in ponds and to determine the effectiveness of a wetland in improving pond effluent quality. This information will be beneficial in verifying whether or not water exchange should be a recommended practice. It also will be beneficial in ascertaining if wetlands should be recommended for treating pond effluents. The research findings and other information on BMPs for pond effluents will be provided to farmers in a workshop. The number of BMPs developed from the research findings can be quantified as can the number of farmers who attend the workshop.

#### Research Design

*Location:* The study will be conducted at the Centro de Aqüicultura, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil.



## **Methods**

*Pond facilities:* Six earthen ponds, three with water exchange of about 10% pond volume per day and three without water exchange, will be used. These ponds are 0.23 to 0.92 ha in area and 1.2 m deep. The wetland is a shallow, 80 m long ditch with dense populations of *Typha* sp., *Eichhornia crassipes*, and other macrophytes.

*Culture period:* June 2003 to March 2004.

*Fish species:* Nile tilapia, *Oreochromis niloticus*.

*Stocking rate:* Two fish m<sup>-2</sup>.

*Nutrient inputs:* Inorganic fertilizer and feed.

*Water management:* Three of the ponds will have water exchange at about 10% pond volume per day. The other three ponds will be operated without water exchange, but seepage and evaporation losses will be replaced. The effluent water from the ponds with water exchange and the draining effluent from all ponds will pass through the wetland.

*Sampling schedule:* At 2-week intervals, water samples will be collected from the water supply for the six ponds and from sampling points near the water outlet in each pond. One water sample will be taken from the influent to the wetland and another from the effluent of the wetland at two-week intervals.

*Water analyses:* The variables to be analyzed are listed below:

<b>Variables</b>	<b>Method</b>
Residence time	Volume
Water transparence	Secchi disk
Dissolved oxygen	Winkler method, Golterman et al. (1978)
Temperature	Corning PS 16 thermometer
PH	Corning PS 15 pH meter
Electrical conductivity	Corning PS 17 conductivity meter
Total alkalinity and inorganic carbon	Mackereth et al. (1978)
Ammonia	Koroleff (1976)
Nitrite, nitrate, Total phosphorus and ortho-phosphate	Golterman et al. (1978)
Chlorophyll <i>a</i>	Nush (1980)
Total dissolved solids	Boyd and Tucker (1992)
BOD (Biological Oxygen Demand)	Boyd and Tucker (1992)
Hardness	Boyd and Tucker (1992)
Total suspended solids	Boyd and Tucker (1992)

*Statistical analysis:* The null hypotheses to be tested are as follows:

- 1) The fish yields from the static ponds are no different than those of the ponds with water exchange.
- 2) Concentrations of water quality variables are not different between the two treatments.
- 3) The concentrations of water quality variables are less in wetland effluent than in wetland influent.
- 4) Water quality does not differ between source water for culture ponds and wetland effluent.

Statistical analyses of water quality parameters and fish yields will include calculations of standard errors for means and comparisons of means with Duncan's multiple-range tests ( $\alpha = 0.05$ ). Analyses will be performed with Sigma Stat for Windows (SPSS, 1997).

### Regional Integration

This experiment addresses a key aspect of overall CRSP objectives, which is to develop sustainable aquaculture through better understanding of system dynamics and development of better practices that individual farmers can use to guide their individual farming operations.

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## Pond Design and Watershed Analyses Training

Water Quality and Availability 3 (11WQAR3)/Study/Honduras, Panama

### Investigators

Dan Meyer	HC Principal Investigator	Escuela Agricola Panamericana, Zamorano, Honduras
George Pilz	HC Co-Principal Investigator	Escuela Agricola Panamericana, Zamorano, Honduras
E. William Tollner	US Principal Investigator	University of Georgia

### Objectives

- 1) Finish the translation of existing pond models (developed during Work Plan 10) to Spanish.
- 2) Implement an improved web-based delivery approach for the design and economics analyses models as one part of a major cross cutting project activity relating to a comprehensive web-based internet delivery system for trainers and connected farmers.

### Significance

The hillsides of Latin America cover about 1 million km<sup>2</sup> and provide livelihood for some 20 million people, among whom roughly half are classified as “poor” and live in marginalized, rural communities (Knapp et al., 1997). Principal Central American countries (followed by percent area of steep-slope agriculture) are: Honduras and Nicaragua (80%), Costa Rica (70%), and El Salvador and Guatemala (75%) (CIAT, 1996). Typically, the hilly landscape is very heterogeneous and made up of small plots. About half of the hillside ecosystem in Central America is progressively deteriorating due to the combined effects of deforestation, overgrazing, destructive tillage techniques, improper water management, and unfavorable socioeconomic conditions (Whiteford and Ferguson, 1991; Knapp et al., 1997). This has serious implications for agroecological sustainability.

Together with other watershed management initiatives (e.g., soil conservation measures, agroforestry, etc.), pond aquaculture can play an important role in stabilizing these ecosystems (Scherr and Yadav, 1997) as testified by Asian experiences (e.g., Nepal, the Philippines, etc.). Fishponds also serve multiple roles including water conservation, income generation, and food production. However, hillside ponds are rare in Central America apparently because of high costs associated with mechanized earth moving and/or high labor needs for hand construction, and lack of knowledge of alternate designs suited to local conditions. Further, research by both Zamorano (Lee, 1997) and CIAT (1997) suggests that poor understanding of biophysical (landscape) and socioeconomic (lifescape) linkages among farmers in hillside watersheds impedes more sustainable use of land and water resources in Honduras. Our experiences in Honduras confirmed these impressions. We found that our pond design training was well received in Honduras and has beneficial potential for all of Central America. Verma et al. (2000, 2001) and Tollner (2001) summarize the progress made on the levee and hillside pond models.

Bringing newly developing countries into the computer age is a daunting task. In the last Work Plan, we evaluated the strategy of placing the levee and hillside ponds (English versions) on a server located at Red de Desarrollo Sostenible (RDS) in Tegucigalpa. The models received substantial hits. For a user to benefit from the models, it was required to log onto the server from the user’s remote location and then download the model to the user’s computer. This download process was lengthy and likely diminished interest due to the size of the models. After evaluating RDS as a web development/host option, we have decided to focus the web development role at Zamorano, where we can build upon a stable infrastructure, interested investigators, dedicated Information Technology and other faculty personnel, and excellent student resources.

With either of these approaches, the models would reside only on one platform, where higher quality program maintenance is possible. The logical next step is to implement this approach in computer labs

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at selected institutions in Central America, beginning at Zamorano.

The core of this activity is to:

- 1) Complete the modeling package in Spanish and English by adding economic analyses to assess various pond designs.
- 2) Replicate the success in Honduras in other Central American countries such as Panama and Mexico, while further enhancing computer infrastructure at selected institutions.

### **Quantified Anticipated Benefits**

Expected benefits include an improved understanding of biophysical and socioeconomic linkages between aquaculture and the associated watersheds, which has implications for sustainable resource management. The work will also help to document perspectives of farmer communities with regard to the role of aquaculture in the agroecosystem(s), which may provide insights into better ways of introducing technology. An indirect benefit is the training (with elements of natural resource planning, social perspectives of resource use, and agricultural-aquacultural interactions) that will be imparted to Zamorano staff. Zamorano will benefit greatly by seeing and obtaining advanced software for teaching and training laboratories.

The lack of interdisciplinary training has been identified as a major weakness of the National Agricultural Research System (NARS) in Honduras (Contreras, 1992). We anticipate that this activity will build on the overall effort to establish Zamorano as a recognized center for aquaculture in Central America. Further, application of engineering principles for assessment of soil and terrain characteristics and water availability in the hillsides will likely lead to more robust pond designs, which in turn has broad applications in Honduras and other parts of Central America.

As a major cross-cutting activity in this overall project, Zamorano plans to actively pursue initiatives with web development, which began in the past work plan. This will solidify Zamorano as a significant presence in web development and web-centered educational initiatives in the Central American region. They will also benefit from exposure to and guidance from the UGA New Media Institute and other experts in web development.

### **Activity Plan**

*Location of Work:* A major portion of the work will be undertaken at Zamorano and at UGA campuses where additional facilities and expertise are available. The NetMeeting Software would be evaluated in a lab at Zamorano and, as experience was accumulated, migrate outward to other Central American countries, including Panama and possibly Mexico.

*Methods:* The proposed plan includes the following sequence of tasks:

- 1) Calculations will be made for embankment stability based on soil characteristics (since design of pond structures depends highly on local topography and soil characteristics).
- 2) Using bracketed values of storage requirement and given topography, modeling tools will be used to bracket embankment volumes needed to create hypothetical storage requirements.
- 3) Efficacy of the pond designs for the intended uses will be evaluated by considering the resulting depths and other geometrical characteristics, storage volume and useful life from sedimentation considerations.

Work will be performed by in-country personnel who have been trained as part of the previous work plan.

**Documentation and Delivery of Pond Designs:** The pond designs developed will build upon past discussions with farmers in Honduras and neighboring countries. In collaboration with Zamorano and other institutions, we will also pursue the possibility of seeking resources to build one or more ponds provided all parties are agreeable. Workshops and training sessions are the primary delivery approaches. Copies of all handouts will be available in Spanish for workshop attendees. We

anticipate two training sessions by host country collaborators for 20 local non-governmental organization representatives, university faculty, and interested farmers in Honduras, Panama, and possibly Mexico.

*Information Technology Transfer:* A two-prong approach is envisioned for technology transfer. We envision the conversion of the Excel®-based models to an appropriate web-enabled format. Some discussion of this was completed this past year when Jennifer Maldonado was in Honduras for training sessions this past spring. We propose to bring their IT person to Georgia for a seminar in advanced web delivery methods. We anticipate this person becoming a regional expert on the use of web technology for information delivery who would spawn future trainings as infrastructure development progressed in the region. The cross-cutting component of this overall project will not only web-enable the pond models but facilitate web delivery of comprehensive tilapia production and marketing information to trainers and connected farmers.

### **Regional Integration**

We plan a formal integration of this activity with the overall region. The work will be jointly conducted by Zamorano personnel (faculty, staff, and students). In-country personnel (Dan Meyers and George Pilz) will assist in organizing training sessions in selected Central American countries.

Much progress has been made with the development of web-based materials. This activity will continue via a cross-cutting activity within this overall project. Personnel at Zamorano have developed excellent interest and capability in serving as web developers. We anticipate bringing their chief IT person to Athens for advanced training at the UGA New Media Institute. We envision this person, working with Dan Meyer and Suyapa Meyer, to serve as a platform for replicating his experience with colleagues at institutions in neighboring countries including Mexico. A cornerstone of our activity will build on experiences learned as we conclude the present Work Plan with training exercises in El Salvador and Nicaragua.

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## Influence of Daily Feed Allowance on Pond Water and Effluent Quality

Water Quality and Availability 4 (11WQAR4)/Experiment/South Africa

### Investigators

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Claude E. Boyd	US Principal Investigator	Auburn University
Lourens de Wet	HC Co-Principal Investigator	Stellenbosch University, Stellenbosch, South Africa
Chhorn Lim	US Co-Principal Investigator	Auburn University

### Objectives

- 1) Optimize daily feed allowance in tilapia culture to increase efficiency of nutrient use, improve pond and effluent water quality, and maintain good fish production.
- 2) Determine effects of daily feed allowance on pond water quality.
- 3) Determine quality of effluents from ponds with different daily feeding allowances.

### Significance

In the Western Cape Province of South Africa alone, there are more than 2,000 reservoirs with an estimated sustainable production capacity of 6,800 tons of fish per annum. This quantity of fish is equivalent to the per capita fish consumption of 3.6 million people (Brink, 2001). There is a great need for additional sources of protein and for more employment opportunities for rural people in South Africa. Modern practices of integrated water management and the science of aquaculture provide an opportunity for the reservoirs to be utilized for fish farming and to supply protein and employment opportunities for rural people. A recent study by Ntala (2002) suggests that fish farming is the most suitable industry to meet the growing demand for fish and related products in South Africa. Fish farming also has the potential to provide an additional sustainable source of protein and to improve the socioeconomic living standards of the rural people of the region (Callebaut, 2002).

Although unemployment and malnutrition are serious concerns in rural South Africa, efforts to solve these problems must be developed in an environmentally responsible manner. Aquaculture can have adverse environmental effects, and water pollution is one of the most likely negative impacts. As feed is the major source of nutrients causing water pollution, good feed management is of major importance in responsible aquaculture.

The dynamics of pond ecology is complex and difficult to quantify. Salie et al. (1998) found that the concentrations of certain water quality variables in irrigation dams fluctuate seasonally. The existing small-scale fish farming projects are all semi-intensive and intensive production systems. The fish species are fed commercially formulated rations. Ponds are normally stocked with small fish and feeds are applied daily at a certain percentage of the weight of the fish in the pond (Boyd, 1990). Feeding rates are adjusted periodically for weight gain by fish. The negative impact of poorly managed aquaculture systems on the environment, and particularly on water quality, is becoming an important issue and constraint to aquaculture expansion. The main input in most intensive fish culture systems is feed, which is partly transformed into fish biomass and partly released into the water as feces or as unconsumed feed. Although aquaculture feed is a source of nutrients for fish, it can also be the major source of pollutants. Nitrogen, phosphorous and organic substances (from unconsumed as well as undigested feed) are the main factors affecting environmental pollution. As the stocking density is increased, a proportionate amount of metabolic wastes is produced. Accumulation of organic substances and metabolic wastes may lead to toxicity and suppression of growth in fish. It also may lead to the release of harmful gases, such as hydrogen sulfide and methane from sediment, that can be a significant cause of stress and health risk. For fish farmers it is necessary to maintain good water quality throughout the production system. Wastewater discharged from poorly managed intensive

aquaculture systems may lead to deterioration in water quality resulting in depletion of dissolved oxygen, hyper-nutrication, and discharge of organic matter. Specifically, wastes from overfeeding are pollutants. The phosphorous and nitrogen present in the feed are especially troublesome by causing eutrophication of receiving waters. Depending on the species and culture techniques, up to 85% of phosphorus and 50 to 90% of nitrogen input into a fish culture system as feed may be lost into the environment through uneaten feed, fish excretion, feces, and respiration (Wu, 1995).

A factor in favor of aquaculture development in South Africa is the New Water Act, which encourages optimal and sustainable use of available water bodies (Brink, 2001). In order to reduce the possible negative impact of aquaculture, governments of several countries are already adopting policies to reduce the pollution of aquatic environments by aquaculture effluents and stressing the need for research on this topic (Gonzalez et al., 1996; Twarowska et al., 1996; Easter et al., 1996; Cornel et al., 1993; Suvapepun, 1994). The approach towards optimizing feeding practices should be a proactive one ensuring environment-friendly aquaculture opportunities for integrated water use.

The basic understanding of feeding practices is of prime importance to fish farmers in determining optimal feeding regimens, meal sizes, feeding frequency, and duration of feeding cycles—all that should regularly be adjusted in order to optimize growth and feed efficiency. Better feeding practices will lead to an improvement in pond and effluent water quality. Farmers lacking experience in this field often fail to do just that.

The research described in this proposal fits well under the Eleventh Work Plan Research Themes. The proposal falls under Production System Design and Integration and it focuses on the themes of fish nutrition and feed technology, and water quality and availability.

### **Anticipated Benefits**

*Target Groups:* Our target groups are peril-urban and rural communities with limited or no access to aquaculture inputs (water bodies, finances, expertise, etc.).

*Quantifiable Direct Benefits From Research and Outreach Work:*

- 1) Establish relationships between feed allowance and pond water and effluent quality.
- 2) Verify whether or not reducing feed allowance slightly for improving water quality will lessen fish production.

*Quantifiable Indirect Benefits From Research and Outreach Work:*

- 1) Optimal feed allowances for ponds that cause minimum negative environmental impacts.
- 2) Critical concentrations of important water quality parameters.
- 3) Seasonal trends in water ecology.
- 4) Develop feed management for optimal nutrient utilization in pond culture systems.

### **Research Design**

*Location:* Elsenburg Research Farm, Western Cape Province, South Africa. It is located 50 km northeast of Cape Town.

*Methods:* Earthen ponds will be used to investigate the effect of feeding level and frequency on pond water and effluent quality. Feeding allowances will be 70, 85, and 100% of apparent satiation. The following pond water quality variables will be measured: dissolved oxygen (daily), pH, ammonia, nitrite, nitrate (weekly), total phosphorus, total nitrogen (every two weeks), alkalinity (monthly), turbidity, and chlorophyll *a* (every two weeks). Natural productivity will be assessed by turbidity and chlorophyll *a* measurements at two-week intervals. Growth of fish will be assessed by samples taken at four-week intervals. At harvest, water quality variables indicated above will be measured in effluents. Fish will be weighed and samples analyzed for dry matter, nitrogen, and phosphorus. The amounts of nitrogen and phosphorus applied in feed, removed in fish, and discharged in effluent at harvest will be estimated for each feeding regime.

*Pond Facilities:* 10 earthen ponds (20 m × 10 m × 1.25 m = 250 m<sup>3</sup>).

*Culture Period:* 6 months (October to March).

*Fish Species:* Mozambican tilapia (*Oreochromis mossambicus*).

*Stocking Rate:* 8 to 15 kg m<sup>-3</sup>.

*Nutrient Inputs:* Commercially available tilapia grower feed.

*Water Management:* Earthen ponds, no aeration, natural water replacement.

*Sampling Schedule:*

- Water quality analyses (daily/weekly/twice-weekly/monthly) depending upon the variable (see research plan).
- Fish sampling and weighing (every four weeks).
- Effluent and fish composition samples (at harvest).

*Statistical Analysis:* A completely randomized design (CRD) will be used. Effect of feeding levels on pond water and effluent quality will be done with three replications of each of the following treatments: 100% of apparent satiation; 85% of apparent satiation; 70% of apparent satiation, twice daily. Null hypothesis: Decreasing feeding levels improves pond water and effluent quality without affecting fish growth performance.

### **Regional Integration**

*The findings of this research will be presented to the following organizations:*

- International and local commercial feed companies.
- Centre for Scientific and Industrial Research (water analyses).
- Agricultural Research Council: Engineering (system design).
- Hands-on Fish Farmers Co-operative (small-scale fish farmer representatives).
- Departments of Animal Sciences and Genetics, University of Stellenbosch (statistical analyses).

These organizations will assist with dissemination of the research findings to farmers.

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## Preliminary Work on Site Description, Evaluation, and Development Planning: Tanzania, Ghana, and Kenya

Economic/Risk Assessment and Social Analysis 1 (11ERAR1)/Activity/Tanzania, Ghana, and Kenya

### Investigators

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Ben Ngatunga	Collaborator	Kingolwira National Fish Farming Center, Ministry of Agriculture, Tanzania
Raphael Mapunda	Collaborator	Fisheries and Aquaculture Development Division, Ministry of Agriculture, Tanzania
Stephen Amisah	Collaborator	University of Science and Technology, Ghana
Linus Kumah	Collaborator	Fisheries Department, Ministry of Food and Agriculture, Ghana

### Objectives

- 1) Verify the existence of basic institutional research capacity needed to make collaborative research viable in Kenya, Tanzania and Ghana.
- 2) Secure and study government policies to ascertain support for aquaculture, research, and extension.
- 3) Determine the host government's interest in participating in PD/A CRSP programs and its willingness to make adequate commitments to support various research activities.
- 4) Determine the interest and commitment of the host country PIs to PD/A CRSP programs and research activities.
- 5) Establish a mutual understanding of responsibilities and the expectations of PD/A CRSP from host countries.

### Significance

The PD/A CRSP has a global and a regional focus to aquaculture research with a goal of promoting economic growth, enhancing food security, and conserving natural resources in developing countries. Consequently, PD/A CRSP continues to welcome regional and site-specific research to complement its experiments. In Africa, Kenya is the only country actively involved in PD/A CRSP activities. The work in Kenya, particularly research activities at the Sagana Fish Farm have attracted scientists from neighboring countries including Uganda, Malawi, and Tanzania. Through the PD/A CRSP program, Moi University in Eldoret, Kenya currently has an active fisheries and aquaculture teaching, research and extension program.

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Under Work Plan 7, Tanzania and Ghana were among countries that were evaluated for possible collaborating sites for PD/A CRSP activities. This proposal incorporates input from Work Plan 7 to evaluate the current potential for regional outreach in Tanzania and Ghana, as well as markets for aquaculture products in the Eldoret area in Kenya. Currently, Sokoine University of Agriculture (SUA) in Tanzania reports a developed aquaculture program. The Animal Science and Production Department of SUA has an undergraduate program in fisheries and aquaculture. Facilities include 20 concrete tanks each with a surface area of 7 m<sup>2</sup>, and ten earthen ponds each with surface area of 600 m<sup>2</sup>. The department has a joint research project with the Tanzania Fisheries Research Institute (TAFIRI) on evaluation of locally available feedstuffs as feed resources for fish, as well as investigating the possibility of culturing endangered indigenous species of Lake Victoria fish, e.g. *Oreochromis variabilis*.

In Ghana, the Kwame Nkrumah University of Science and Technology (KNUST) has a department of Freshwater Fisheries and Watershed Management that is actively involved in aquaculture. The department reports considerable strengths and expertise in various aspects of freshwater fisheries and aquaculture including water bioresources and aquaculture, fish nutrition, fish reproduction, fish and gear technology, hydrobiology, aquatic pathobiology, and aquatic pollution. The Department has teaching and research programs at undergraduate and postgraduate levels. Graduates from the department have undertaken post-graduate programs at UAPB, Arkansas during the past three years. The KNUST facilities include 16 fish ponds, a laboratory building, and a hatchery at the research farm station.

This activity proposes to visit Tanzania and Ghana to evaluate developments in aquaculture programs and to achieve the objectives indicated above. In addition, the visit will ascertain compliance of the two potential new host countries with BIFAD guidelines and suitability as prime research sites. The activity will also involve a visit to Kenya to study market development strategies for the Eldoret area where the influence of the aquaculture program at Moi University is promoting fish farming. The Eldoret area is a traditional beef-consuming region.

### **Quantified Anticipated Benefits**

The activities in Tanzania and Ghana will provide data and information on existing physical infrastructure such as ponds, suitable land for pond construction, water supply, feed availability, fish species availability, laboratory facilities, communications, housing, transportation, and road access. In addition, information relating to health, safety, and adequate working conditions for the successful completion of research projects will be provided. We will also be able to provide assessments of operational costs, capital costs, and other costs that would be associated with a potential research project.

Various meetings with potential research personnel will provide assessments of the willingness, knowledge, skill, and general caliber of research personnel, including PIs, support staff, and undergraduate and graduate students. From these meetings, we will develop a documentation of mutual understanding and agreements relating to responsibilities and the expectations of the PD/A CRSP from the host countries. This documentation will also include a template of a reporting system to assess the progress of the collaborative efforts.

A site report for Ghana and Tanzania that entails all the above will be written with other information pertaining to the overall aquaculture development potential in the country, extension apparatus, and government policies or strategies that may affect the efforts of the PD/A CRSP and in particular, the conduct of research and dissemination of results.

In Kenya, the activity will provide information on the process by which area citizens could engage in fish marketing and a recommendation of a framework for developing necessary capacity building and market organization for fish marketers.



### Activity Plan

- 1) Establish site evaluation criteria with input from study C, Work Plan 7, PD/A CRSP new host country research location considerations, and the BIFAD guidelines.
- 2) Gather site information on government policies, political climate, business conduct, extension network, and other technical information through interviews, government publications, USAID missions, and other sources.
- 3) Visit the various universities in the host countries, as well as government research stations with the evaluation criteria as a template for gathering information.
- 4) Visit area fish farms and market and meet with fish farmers.
- 5) Hold discussions with university professors, government officials, and other relevant personnel.

### Training Local Farmers on Safe Handling of Steroids and Masculinization Techniques in Central America

Applied Technology and Extension Methodologies 1 (11ATER1)/Activity/Mexico

#### Investigators

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Eunice Perez-Sánchez	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco
Carl B. Schreck	US Principal Investigator	Oregon State University
Guillermo R. Giannico	US Co-Principal Investigator	Oregon State University

#### Objectives

- 1) Conduct three regional workshops on safe handling of steroids and masculinization techniques in Mexico.
- 2) Generate printed and electronic materials for safe handling of steroids and masculinization techniques.

#### Significance

Training of extension agents, farmers and students is considered to be an important activity by the PD/A CRSP. The need to deliver recently generated information and technological packages to the immediate users is fundamental for aquaculture development. Training workshops are one way to achieve these goals. Through workshops, researchers can obtain feedback information from farmers and identify problems that may compromise advances in the field of interest. According to the PD/A CRSP Training Plan: Perspective, Experiences, and Directions, the training of technicians, farmers, and extension agents can be the most effective way to disseminate research results and good practices in aquaculture (Bolívar et al., 2002).

The administration of natural and synthetic steroids during early development of fish has been successfully used to induce sex inversion in several species (see reviews by Schreck, 1974; Hunter and Donaldson, 1983), and has become a common practice in the production of single sex populations to enhance productivity in the aquaculture industry. Among the techniques developed, oral administration of steroids via feeding has become the most commonly used. In tilapia culture, the production of all-male populations through treatment of fry with 17 $\alpha$ -methyltestosterone (MT) impregnated food has become the most widely used procedure. Other readily available anabolic steroids (such as fluoxymesterone) are also used by farmers who exercise little or no precaution concerning exposure to the compounds. Despite the success of this masculinizing technique, significant "leakage" of MT into the pond environment may occur from uneaten or unmetabolized food. This leakage poses a risk of unintended exposure to anabolic steroids by hatchery workers as well as fish or other non-target aquatic organisms. Furthermore, in some countries, pond sediments are dredged and sometimes used to prepare soil for crop production, thereby spreading the risk of exposure to MT to terrestrial systems and to other aquatic systems (Contreras-Sánchez, 2001).

Despite the wide use of MT for masculinizing tilapia in aquacultural facilities, few efforts have been devoted to eliminate this steroid from farm effluents. Recently, several institutions in the US have combined efforts to provide information needed by the FDA to gain MT use approval for aquaculture (Green and Teichert-Coddington, 2000). These efforts are focusing on maintaining low levels of MT in the water, instead of eliminating it completely. The problems associated with contamination of water and sediments are further compounded by the many effects related to bioaccumulation and the transfer of the contaminants and their metabolites through the food web (Kime, 1998). Therefore, it is important to promote the safe use of MT and other steroids in aquacultural facilities by incorporating preventive measures such as filtration, biodegradation, or photodegradation of the steroid and its metabolites.

Aquaculture systems worldwide have been responsible for severe environmental degradation. Producing clean farm effluents through environmentally sound technology (such as charcoal filtration or photodegradation) may be a means of reducing negative impacts on the environment.

Developing new techniques for production of clean effluents would be futile unless the information that is generated is transferred to people conducting aquacultural activities. This is especially difficult in Mexico and Central America because information is not readily accessible. Workshops conducted in Mexico under CRSP support have already impacted tilapia culture in Tabasco and Chiapas and most farmers are growing sex-reversed tilapias—this activity was not conducted until only a few years ago. To complement research for the production of clean sex-inversion techniques, we believe that it is of vital importance to train farmers and extension agents and provide printed materials for the safe handling of steroids in aquacultural facilities.

### **Anticipated Benefits**

Workshops conducted in Central America will educate extension agents, technicians, and farmers on safe and effective sex inversion techniques. These personnel can then train additional growers. A manual (both hard copy and web-based) in English and Spanish will be produced for dissemination to high school and university students as well as the personnel listed above.

### **Activity Plan**

#### **Activity 1: Production of a Manual for Sex Inversion Techniques, Safe Handling of Steroids and Treatment Methods for Effluents.**

The following is a general outline for the manual:

Biology of fishes used in aquaculture in Central America

    Growth and feeding

    Reproduction

    Diseases

Culture Systems

    General aspects

    Fry production

    Onset of feeding

    Grow-out

    Pond culture/cage culture

Control of sex

    Hand sorting

    Sex inversion with steroids

    Oral administration

    Immersion

    Hybridization

    Production of YY males

Safe handling of steroids

Incorporation of steroids into food

Working with water containing steroids

Treatment of effluent water

    Charcoal filtration

    Photodegradation

*Schedule:* Manual preparation, May 2003–April 2004; Technical report, 30 April 2004.

#### **Activity 2: Incorporate the Manual from Activity 1 into a Website.**

Design, implementation and maintenance of the website will be conducted at UJAT. These services will be provided by the university at no cost.

*Schedule:* Website design and implementation, May 2003–April 2004; Technical report, 30 April 2004.

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### Activity 3: Produce posters that demonstrate safe handling of steroids and place them at aquacultural facilities.

Approximately 300 posters will be produced. Posters will be distributed at workshops and mailed to appropriate aquaculture agencies, farmers, educational institutions and extension personnel (see Activity 4).

*Schedule:* Poster design and printing, May 2003–April 2004; Technical report, 30 April 2004.

### Activity 4: Conduct Three Workshops in Mexico Concerning Sex Inversion Techniques, Safe Handling of Steroids, and Treatment Methods for Effluents.

The first will be conducted in Villahermosa, Tabasco and will cover the southeastern region. The second will be held in Mazatlán, Sinaloa and will be organized in coordination with CIAD. This workshop will cover the Pacific region. The last workshop will be conducted in Mexico City and will be organized in coordination with Universidad Autonoma Metropolitana-Xochimilco. This workshop will cover the central region. The materials produced in the first workshop will be used to implement the second and third ones. A key player in the organization of these workshops will be the Aquaculture Directors of the National Fisheries Institute, which has contacts with tilapia farmers all over the country.

Officials and universities will be contacted to create a network of farmers who are interested in participating. If necessary, we will conduct workshops in outlying areas. Nongovernmental organizations involved with farmers will be contacted to help create the workshop network. They will play an important role for local organization.

### Regional Integration

We will exchange information with Dr. Daniel Meyer, at the Pan-American Agricultural School. If these activities are successful, we will contact other CRSP research teams to exchange information and provide the materials generated in this investigation.

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## **Aquaculture CRSP Sponsorship of the Sixth International Symposium on Tilapia in Aquaculture**

Applied Technology and Extension Methodologies 2 (11ATER2)/Activity/Global

### **Investigators**

Remedios Bolivar	HC Principal Investigator	Central Luzon State University, Nueva Ecija, Philippines
Kevin Fitzsimmons	US Principal Investigator	University of Arizona

### **Objectives**

- 1) Provide travel support for an organizational meeting to sign contracts for the Sixth International Symposium on Tilapia (ISTA 6) in the Philippines.
- 2) Help support attendance for Dr. Remedios Bolivar and three international contributors from other Aquaculture CRSP countries to attend ISTA 6.
- 3) Employ a CLSU student to assist with the compilation of papers submitted to the ISTA 6 conference proceedings.

### **Significance**

The ISTA meetings are held every four years and are the premier international meeting focused directly on tilapia aquaculture. In the past the ISTAs have provided one of the most important outlets for publication and discussion of the findings of Aquaculture CRSP supported research. The Aquaculture CRSP has been a co-sponsor of the last two ISTAs (Fitzsimmons, 1997; Fitzsimmons and Carvalho, 2000). CLSU will be hosting ISTA 6, and we will be especially interested to ensure the success of the conference.

Travel support is critical to the ability of scientists from developing countries to present their findings in international fora. Host country CRSP scientists benefit from the opportunity to discuss their work amongst themselves, with their US colleagues, as well as with the rest of the international community.

The efforts within this proposal fit the Activity description of conference organization.

### **Anticipated Benefits**

Target groups:

- 1) Host country scientists:
  - Our target group is the international community devoted to tilapia aquaculture.
  - Partial financial support for a graduate student to assist with conference planning.
- 2) Direct benefits from the conference:
  - Organize and host the Sixth International Symposium on Tilapia in Aquaculture.
  - Ensure a strong international participation in ISTA 6.
  - Present and publish a large body of information generated by Aquaculture CRSP researchers.
- 3) Indirect benefits from the conference:
  - Establish relationships between tilapia research community and local Filipino farmers.
  - Have a strong program with informative sessions and good networking.

### **Research Design**

*Location:* ISTA 6: CLSU and Manila Conference Center.

*Methods:* Fitzsimmons and Bolivar are on the organizing committee planning the ISTA 6 symposium.

A subcommittee will be formed to set the selection criteria and then determine which applicant

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scientists will be awarded the travel support. The selection criteria will be based on contributions of papers to the conference, past participation in Aquaculture CRSP projects, and other available support. If partial support can be generated from other sources, the funds may be split to support additional participation.

A graduate student from CLSU will be hired part-time to assist with the conference organization. Specifically, the student will work with the publication committee working on the proceedings.

### **Regional and Global Integration**

- Local participation on organizing committee.
- Regional participation on organizing committee.
- International participation on organizing committee.
- Local, regional, and international co-sponsors and vendors for trade show.
- Strong international representation in papers presented.

### **Literature Cited**

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## **Aquaculture CRSP—Global Contributions to Sustainable Aquaculture: A Special Session at the 2004 World Aquaculture Conference**

Applied Technology and Extension Methodologies 3 (11ATER3)/Activity/Global

### **Investigator**

Kevin Fitzsimmons                      US Principal Investigator                      University of Arizona

### **Objectives**

- 1) Organize a special session at the 2004 World Aquaculture Conference meetings that will focus on Sustainable Aquaculture Research conducted in Aquaculture CRSP countries.
- 2) Provide travel support for three or more international contributors from the Aquaculture CRSP to attend the 2004 World Aquaculture Conference and participate in the special session.
- 3) Provide three poster and/or presentation awards for CRSP-funded students.

### **Significance**

Every third year the World Aquaculture Society holds a “triennial” meeting in the United States. These meetings are co-hosted by the American Fisheries Society and the Shellfish Society. The 2004 meeting will probably be the single biggest aquaculture meeting ever held. The Aquaculture CRSP will sponsor a special session focused on research conducted by Aquaculture CRSP scientists. Findings from this research will benefit fish producers in developing and developed countries.

Travel support is critical to the ability of scientists from developing countries to present their findings in an international forum. Host country CRSP scientists benefit from the opportunity to discuss their work amongst themselves, with their US colleagues, as well as with the rest of the international community.

The effort within this proposal fits the Activity description of conference organization.

### **Anticipated Benefits**

Target groups:

- 1) CRSP scientists:
  - Our target group is the international community devoted to development of sustainable aquaculture technologies in developing countries.
- 2) Quantifiable direct benefits from the conference:
  - Have a special session at the World Aquaculture Conference that presents a comprehensive overview of the research contributions of the Aquaculture CRSP.
  - Ensure a strong participation of Aquaculture CRSP Principal Investigators in the World Aquaculture meetings.
  - Present and publish a large body of information generated by Aquaculture CRSP researchers.
- 3) Quantifiable indirect benefits from the conference:
  - Establish relationships between the CRSP research community and international aquaculture scientists, producers, and development community.
  - Have a strong program with informative presentations and good networking.

### **Research Design**

*Location:* WAS 2004, Arizona and Honolulu, Hawaii

*Methods:* Fitzsimmons has already been asked by the program committee planning the conference to organize the session. The session has been approved and is listed in the initial program and call for papers.

A committee will be formed to determine the selection of visiting scientists who will be awarded

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the travel support. The selection criteria will be based on contributions of papers to the conference, past participation in Aquaculture CRSP projects, and other available support. If partial support can be generated from other sources, the funds may be split to support additional participation.

A committee will be formed to determine the selection of students who will receive awards for their WAS 2004 poster or paper. Students that were funded on the current four-year Aquaculture CRSP grant will be eligible for the award.

### **Regional and Global Integration**

Local participation on organizing committee.

Regional participation on organizing committee.

International participation on organizing committee.

Local, regional, and international co-sponsors and vendors for trade show.

Strong international representation in papers presented.

### **Literature Cited**

<[www.was.org](http://www.was.org)> Program and call for papers website.

**Aquaculture Training for Kenyan Fisheries Assistants**

Applied Technology and Extension Methodologies 4 (11ATER4)/Activity/Kenya

**Investigators**

Charles C. Ngugi	HC Principal Investigator	Moi University
Bethuel Omolo	HC Co-Principal Investigator	Fisheries Department, Government of Kenya
Christopher Langdon	US Principal Investigator	Oregon State University
James Bowman	US Co-Principal Investigator	Oregon State University

**Objective**

To provide in-service training for Kenyan Fisheries Assistants to enable them to disseminate appropriate current aquaculture information to fish farmers

**Significance**

The issues of food security and fish production continue to be of great importance in Kenya. This proposal complements previous PD/A CRSP efforts to address these issues through appropriate training for personnel involved in aquaculture extension activities, namely Fisheries Assistants and Fisheries Officers of the Kenya Fisheries Department (FD).

As in many developing countries, food security is a critical issue in Kenya, and maintaining an adequate and sustainable supply of fish and other aquatic products is an important part of this issue. High quality products should be accessible to the consumer in sufficient quantity to provide adequate nutrition. There is no doubt that in Kenya fish can contribute to food security directly by making high quality food available to communities and indirectly by providing incomes and livelihoods to the producers and fish farmers.

Since we are near a state of crisis with respect to food supplies in Kenya, the future contribution of fish to food security is in question and now is an appropriate time to examine fish production issues. Past experience shows that fisheries resources in Kenya have been degraded and overexploited due to a lack of qualified managers and uninformed stakeholders. The end results are drastic reductions in fish catches and huge post-harvest losses, leading to the supply of poor quality fish to the markets or no supply at all. Over the next 25 years, the challenges in fisheries management will be to maintain fish harvests at present or near-present levels while sustainably increasing aquaculture production to meet demand for fish and other aquatic resources. The Government of Kenya’s National Development Plan (1996) called for an increase in aquaculture production to compensate for the shortfall in production from capture fisheries.

Increasing aquaculture production in a sustainable way requires trained manpower. Training opportunities in the past have been limited. Sustained funding and well-thought-out training strategies have been lacking. In the decade prior to the beginning of PD/A CRSP efforts in Kenya, Fisheries Extension Officers serving in the Government had received no formal training because the only training institution, the Naivasha Wildlife and Fisheries Training Institute, reverted to training Wildlife Officers only. This had far reaching repercussions for the fisheries sector, because serving Fisheries Officers and Fisheries Assistants, who are charged with the responsibility of managing the country’s fisheries resources and helping fish farmers manage their ponds and farms effectively, had received neither initial basic training nor periodic in-service training.

There has therefore been a need for capacity development through assistance from reputable international institutions. In-service training of Fisheries Officers and Fisheries Assistants by experienced personnel enables them to transfer appropriate knowledge and skills to fish farmers, which is necessary for the continued development of aquaculture in the country. Wangila (1996) stated that

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more technical certificate, diploma, and higher level training should be included in the aquaculture sector's future training plans. This need was recognized in the CRSP's Regional Plan for Africa (PD/A CRSP, 1997), and Provincial Fisheries Officers, officers of the Kenya Marine Fisheries Research Institute (KMFRI), and faculty from Moi University's Department of Fisheries identified training as a primary need in a 1997 workshop held at Sagana Fish Farm (CRSP Workshop, 1997). Planning for such training was begun at that workshop and later incorporated into the CRSP's Ninth and Tenth Work Plans (WP9 and WP10). Feedback from the training sessions conducted under those work plans shows that they have been extremely valuable to the participants, giving them the ability to construct fish ponds properly and to give sound pond management advice to farmers. The FD has been so satisfied with the results of these training sessions that they are now contributing matching funds to support additional training courses. Because of the very positive feedback received from earlier sessions and the FD's willingness to contribute funds to the effort, we propose to conduct two additional training sessions during the next phase of CRSP work (WP11).

### **Quantified Anticipated Benefits**

Fisheries Assistants will learn how to construct fish ponds, how to handle fish, and how to manage ponds and fish stocks properly. Receiving this training will not only increase their skills but will also improve their morale and increase their confidence when advising extensionists and fish farmers. Better-trained officers will improve the fisheries extension services by providing better information to fish farmers. Properly informed farmers will increase production of farmed fish through better fish husbandry. Linkages between researchers and extension workers will be improved and the linkage between the Kenya Fisheries Department and the Moi University Department of Fisheries will be strengthened. Forty Fisheries Assistants who did not receive training during WP9 and WP10 will receive short-term training, and it is expected that the information and expertise they gain will ultimately be disseminated to a considerably wider audience of extension workers and fish farmers. The expertise of Moi University faculty and students involved in the training will be strengthened in both aquacultural and educational techniques.

### **Activity Plan**

The Kenya Fisheries Department has already sponsored a two-week course for Fisheries Assistants (20 participants, April 2003), held at the MU Chepkoilel Campus in Eldoret. This proposal is to match that training session with a like session for an additional 20 Fisheries Assistants. Training will be conducted at either the Moi University Fish Farm, Chepkoilel Campus, Eldoret, or at Sagana Fish Farm, the CRSP prime site in Kenya. Moi University faculty and the US Principal investigator will ensure that course content is up to date. As for previous training courses, this additional session will be evaluated by the Kenya Fisheries Department's Training Assessor. The majority of the training will be done by faculty and other resource persons from Moi University, under the leadership of Dr. Charles Ngugi, with assistance from Sagana Head of Station Bethuel Omolo and US PI Jim Bowman. Course content will include pond construction, soil characteristics and management, fry and fingerling production of various species under culture in the region, and general principles of pond management. Fish handling, pond fertilization, integrated systems, water quality management, fish nutrition and disease control, predators and fish marketing will also be covered, and pond systems will be compared with other, more-intensive types of culture systems. Emphasis will be on small-scale fish farming as a business enterprise. Approximately 2/3 of the time will be spent doing field work and the remaining 1/3 will be spent in a classroom setting. If outside funding is provided, up to five additional trainees will be allowed into the session; these can include trainees from neighboring countries.

### **Regional Integration**

This activity directly addresses Objective 2 of the PD/A CRSP Regional Plan for Africa (PD/A CRSP, 1997) which is "to assist in the development and conduct of aquaculture training courses and programs, with emphasis on pond operation and management." Experience gained since the Regional Plan was drafted has shown the need to include a basic pond construction component in this type of training. Further, the proposed activity addresses the general goals, stated in the Regional Plan, of strengthening research (Moi University) and extension (Kenya Fisheries Department) institutions, and of encouraging collaboration and technology transfer among research and extension institutions.

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### Mitigating the Effects of High Temperature and Turbidity on Seed Production of Nile Tilapia from Hapa-in-Pond Systems

Seedstock Development and Availability 1 (11SSDR1)/Experiment/Thailand

#### Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology, Pathumthani, Thailand
James S. Diana	US Principal Investigator	The University of Michigan
James E. Rakocy	US Principal Investigator	University of the Virgin Islands

#### Objectives

- 1) Increase seed production of Nile tilapia by lowering water temperature through increased pond water depth.
- 2) Improve seed production and water quality by reducing mud turbidity and fouling.

#### Significance

Among several methods used for mass seed production of Nile tilapia (*Oreochromis niloticus*), hapa-in-pond spawning is perhaps the most efficient and widely adopted by commercial hatcheries in Southeast Asia (Little, 1989; Little et al., 1997). The hapa-in-pond hatchery method developed by the Asian Institute of Technology involves broodfish spawning, egg incubation, and fry collection in net cages (hapas) suspended in earthen ponds. Using this technology, a large number of quality tilapia fry can be produced continuously throughout the year in the tropics (Bhujel, 1999). In Thailand, more than 50 million fry are produced monthly with this method.

Reproduction of Nile tilapia can begin at water temperature as low as 20°C (Popma and Lovshin, 1996) with optimum water temperature ranging from 25 to 32°C (Philippart and Ruwet, 1982; Little and Hulata, 2000). The egg production and hatching are adversely affected by high water temperature in ponds (Bevis, 1994). Drastic drops (> 50%) in seed output occur in most tilapia hatcheries in Thailand when water temperature is above 33°C during the hot season (Little et al., 1997; Bhujel, 1999). To facilitate convenient operation for egg and fry collection, hapas are usually suspended 70 to 80 cm deep in a pond of 1 m or less. This shallowness heats pond water dramatically, resulting in critically high temperatures throughout the water column and causing stress to the fish. Tropical ponds with depths greater than 1.3 m often exhibit vertical temperature differential at midday of 3 to 5°C between the upper and lower water column (Diana et al., 1991). Therefore, suspending hapas below 1 m or so would provide considerably cooler conditions for fish to avoid heat stress.

Another problem of shallow ponds is that water is persistently turbid from resuspension of bottom mud as workers frequently wade through the pond for egg collection. Consequently, muddy water decreases oxygen content by reducing light for phytoplankton photosynthesis and also curtails water exchange in mud-fouled hapas. In addition, the resuspended particles may also contaminate eggs, lowering the hatching rate. One way to eliminate this would be to install walkways under water to facilitate egg and fry harvest in deep ponds without stirring up mud.

#### Anticipated Benefits

The improved deep pond approach will increase efficiency for tilapia seed production by the hapa-in-pond method. This will benefit both small-scale farmers and commercial producers in Asia and other countries. As the hapa-in-pond method continues to be used by farmers, these innovations can easily be extended as well by AIT or country extension agents.

#### Research Design

Location: AIT.



## **Methods:**

### Experiment A: Mitigating the Effects of High Temperature on Seed Production of Nile Tilapia from Hapa-in-Pond Systems

*Pond Facility:* One 400 m<sup>2</sup> pond of 1.6 m deep, 9 hapas of 20 m<sup>2</sup> (8 m x 2.5 m) in surface area.

*Culture Period:* Three months.

*Test Species:* Nile tilapia (*Oreochromis niloticus*).

*Stocking Density:* 1.5 females and 1 male tilapia per m<sup>2</sup> in hapas.

*Nutrient Input:* Broodfish will be fed twice daily with commercial pellets (27% crude protein) at 1% body weight per day.

*Water Management:* Water level will be maintained at 1.6 m deep.

*Sampling Plan:* Water quality will be analyzed biweekly according to standard CRSP protocols. Diurnal dissolved oxygen (DO) and temperature measurements will be made twice a week at 0600, 1000, 1400, 1800, and 2200 h at depths of 10, 40, 70, 100, 120, 140, and 160 cm depth in the water both inside and outside hapas.

Fouling material on each hapa will be collected by washing, and dry weight of the material will be estimated at the end of the experiment.

*Seed Production:* Seed will be collected from the mouths of incubating females at five day intervals, and their bulk weight and number of seeds will be recorded immediately after collection. At each seed harvest, five samples of about 40 eggs each will be taken from each pond treatment. These eggs will be examined under a microscope to determine viability and fouling. Stage I eggs (recently fertilized and undeveloped eggs) will be incubated in down-welling incubators for about 8 to 10 hours, then the eggs will be examined microscopically to observe egg development. Embryos reaching the double-layered gastrula stage will be considered viable whereas embryos without the double layer (appearing opaque under the microscope) will be considered inviable. Stage III eggs (five days after fertilization) will be examined at egg harvest by a direct count of live (viable) and dead (inviable) eggs.

### *Experimental Design, Hypothesis, and Statistical Methods:*

The experiment will be conducted in a randomized complete block design using nine hapas suspended in the pond with three hapa replicates for each treatment for two months. This experiment will have three treatments:

- 1) 70 cm water depth in hapas (control).
- 2) 100 cm water depth in hapas.
- 3) 150 cm water depth in hapas.

Underwater walkways will be constructed in the pond to facilitate egg collecting operation.

Null hypothesis: Water depth in a hapa will have no effect on number of viable seeds produced.

Data analysis: ANOVA and regression.

### Experiment B: Mitigating the Effects of Turbidity on Seed Production of Nile Tilapia from Hapa-in-Pond Systems

*Pond Facility:* Six 200 m<sup>2</sup> ponds of 1 m deep; 18 hapas with 20 m<sup>2</sup> (8 m x 2.5 m) in surface area.

*Culture Period:* Three months.

*Test Species:* Nile tilapia (*Oreochromis niloticus*).

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*Stocking Density:* 1.5 females and 1 male tilapia per m<sup>2</sup> in hapas.

*Nutrient Input:* Broodfish will be fed twice daily with commercial pellets (27% crude protein) at 1% body weight per day.

*Water Management:* Water levels will be maintained at 1 m.

### *Sampling Plan*

Water quality will be analyzed biweekly according to standard CRSP protocols. Diurnal DO and temperature measurements will be made twice a week at 0600, 1000, 1400, 1800, and 2200 h at depths of 10, 40, 70, and 100 cm depth in the water both inside and outside of hapas.

Fouling material on each hapa will be collected by washing, and dry weight of the material will be estimated at the end of the experiment.

Secchi disk visibility will be measured daily at 0900 h. Turbidity will be determined as NTU per man-hour based on the number of workers and time spent in the ponds before and after pond work.

*Seed Production:* Same as Experiment A.

### *Experimental Design, Hypotheses and Statistical Methods:*

The experiment will be conducted using a randomized complete block design in six 200 m<sup>2</sup> ponds. Each pond will contain three hapas with three replicate ponds for each treatment for two months. There will be two treatments:

- 1) Without underwater walkway (control).
- 2) With underwater walkway.

Null hypotheses: Underwater walkways will not influence turbidity of pond waters, and differences in turbidity will not influence pond water quality.

Data analysis: t-test.

### **Regional Integration**

This experiment may become a cost-efficient strategy for mass seed production of Nile tilapia and benefit both small-scale farmers and commercial producers in Asia and other countries. The hapa-in-pond system has been extended broadly, and these modifications can also be used in such extension. The developed methods will be disseminated in local languages to local farmers in the region through the AIT Outreach Program.

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### Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras

Seedstock Development and Availability 2 (11SSDR2)/Study/  
Honduras, Guatemala, El Salvador, Nicaragua

#### Investigators

Daniel E. Meyer	HC Principal Investigator	Escuela Agrícola Panamericana, Zamorano, Honduras
Joseph J. Molnar	US Principal Investigator	Auburn University

#### Objectives

- 1) Evaluate fingerling production techniques utilized in reproducing adult fish and in the production of tilapia fingerlings.
- 2) Compare the prices and quality (uniformity of size, color, and sex-ratio) of tilapia fingerlings for sale in Honduras.
- 3) Evaluate the actual production costs and returns for producing tilapia fingerlings in Honduras.
- 4) Identify and disseminate training materials in Spanish on the topics of tilapia reproduction and fry production to local farmers, nongovernmental organization (NGO) extension agents and other persons interested in tilapia culture.
- 5) Develop extension principles and activities that can be used to increase the number of, and improve the performance of, fingerling producers throughout Central America.

#### Significance

The availability of good quality seed continues to be a factor limiting aquaculture development (PD/A CRSP, 2002). The lack of adequate supplies of all-male tilapia fingerlings has been identified as a principal limiting factor to small-scale fish culture development in Honduras (Meyer, 1988; Triminio, 2001). Procuring reliable supplies of high quality seed for stocking local and remote sites is critical for continued development of the industry. A better understanding of the factors that can contribute to stable seed stock quality and quantity for aquaculture enterprises is essential. The purpose of this study is to document and understand the spatial distribution of fingerling producers, how dispersed seed stock production can be encouraged, and to identify ways seed stock production can be better supported through research and extension efforts.

The production of cultured tilapia in Honduras will surpass 10,000 metric tons in 2002. Most of this production will be from two large commercial farms that supply fresh fillets to North American markets. The large commercial farms are self-sufficient in tilapia fingerling production. They occasionally sell excess fingerlings to small-scale producers.

Cultured tilapia for distribution in domestic markets is estimated at approximately 800 metric tons for 2002. Much of this production comes from small-scale and semi-commercial producers located throughout Honduras. These fish farmers often have difficulties in obtaining all-male tilapia fingerlings for stocking their ponds following each harvest due to logistic problems, and many are dependent on subsidies from NGOs for assistance in obtaining fingerlings and other inputs (Meyer, 2001).

There are several private farms, national fish culture stations, and universities in Honduras that specialize in tilapia reproduction and distribution of sex-reversed fingerlings (Green and Engle, 2000). The quality and sales price of fingerlings available in Honduras is variable (Aceituno et al., 1997).

Tilapia culture continues to expand in Honduras. The inadequate availability and poor quality of tilapia seed was identified as a major constraint to fish culture development locally (Aceituno et al., 1997; Triminio, 2001). The lack of information, or limited access to pertinent information in Spanish, and insufficient training opportunities have also been identified as major constraints to improving farmers'

capabilities to culture tilapia more efficiently and profitably (Triminio, 2001).

Small-scale fish farms are widely distributed throughout Honduras. Honduras is primarily mountainous with limited and difficult access to communities via many roads that are useable for only part of each year. This makes the acquisition of tilapia seed difficult for small-scale fish farmers in many parts of the country.

Many small-scale tilapia farmers have been dependent on NGOs that assist them in aquaculture activities to obtain and transport their seed. The development of fingerling production capabilities in additional areas of the country will provide a greater degree of independence for the farmers to obtain seed locally without this NGO subsidy. This should contribute substantially to making tilapia culture more viable and sustainable in rural areas of Honduras.

In addition, production of all-male tilapia fingerlings can be a very profitable business for local fish farmers (Engle, 1986; Popma and Green, 1990). The level of profitability of fingerling production is dependent on utilizing appropriate technologies and the proper management of fish and other inputs (i.e., costs). There is a documented unsatisfied demand for tilapia seed in many areas of Honduras (Triminio, 2001; PD/A CRSP, 2002).

The study will provide information useful for the development of materials in Spanish. We will endeavor to locate existing materials and adapt these works in light of the study's findings and experiences. Such documents, slide presentations, and other teaching tools will be vital for implementation of fingerling producer training courses in Honduras and possibly other countries in Latin America.

### Anticipated Benefits

Direct target groups of the study will be:

- 1) A minimum of ten tilapia fingerling producers (men and women) that will be included in the survey will directly benefit from this work. These fish farmers will be informed of the results of the study and receive our recommendations as to how to improve their production protocols/ techniques and the quality of the fingerlings that they supply.
- 2) Thirty additional fish farmers (men and women) from strategic areas of Honduras will receive technical training in the areas of broodstock selection and management, tilapia reproduction, and production of fingerlings.
- 3) A total of 40 extension agents and farmers from Central America will benefit from technical training in the areas of broodstock selection and management, tilapia reproduction, fingerling production, and marketing.

Indirect target groups will be:

- 1) Approximately 2,000 small-scale tilapia farmers will benefit by having better quality tilapia fingerlings available locally in Honduras.
- 2) The study will provide an opportunity for a Zamorano student to learn about the situation of local fish farmers and disseminate this information to other students at this institution (800) and at technical meetings.
- 3) The study will enhance Zamorano and Auburn's institutional understanding of the status of small-scale tilapia culture in Honduras and our abilities to propose and undertake effective development projects with aquaculture components. Information and training materials derived from this study will be useful for aquaculture development activities in other countries of Central and South America.

### Research Design

A minimum of ten farms that produce and sell tilapia fingerlings will be visited during the period of September 2003 to March 2004 to evaluate the physical facilities (ponds, tanks, etc.) used for tilapia reproduction and fingerling production in Honduras. Semi-structured interviews will be used to obtain information concerning management protocols and techniques for fingerling production and

distribution on each farm.

At each farm a minimum of 1,000 fingerlings will be purchased and transported to Zamorano for evaluation (purchase price, individually counted, uniformity of size, and color). A subsample of 200 of the fingerlings purchased from each farm will be grown to adult size (> 70 g) on the Zamorano campus. The sex of each adult fish will be determined to ascertain the percent of males in each group.

An inventory of NGOs promoting tilapia culture will be expanded. All of the information from the inventory will be stored in an electronic database for analysis, easy access, and use. This inventory will provide a means for identifying and communicating with NGOs working in aquaculture. The NGOs can request training for their staff, send staff and better farmers to workshops on fingerling production techniques, and more effectively connect to the network of expertise and information on aquaculture that is available in Honduras and the region.

### **Regional and Global Integration**

As resources permit, we will seek to further integrate this activity with the efforts of other institutions and NGOs in the region. Responding to opportunities as they arise and finances allow, the work will be jointly conducted by Zamorano personnel (faculty, staff and, students). In-country personnel (Suyapa Triminio Meyer) will assist in organizing training sessions in selected Central American countries.

Much progress has been made with the development of web-based materials. Personnel at Zamorano have developed excellent interest and capability in serving as a web host. We anticipate bringing their information technology person to Athens, Georgia for advanced training at the UGA New Media Institute. We envision this person, working with Suyapa Triminio Meyer, to serve as a platform for replicating his experience with colleagues at institutions in neighboring countries. A cornerstone of our activity will build on experiences acquired as we concluded the Tenth Work Plan with training exercises in El Salvador and Nicaragua.

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## Continuation of a Selective Breeding Program for Nile Tilapia to Provide Quality Broodstock for Central America

Seedstock Development and Availability 3 (11SSDR3)/Experiment/Mexico

### Investigators

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Carl B. Schreck	US Principal Investigator	Oregon State University
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### Objectives

- 1) Obtain broodstock from an F4 generation of wild and introduced stocks of Nile tilapia based on traditional genetic selection.
- 2) Provide tilapia fry farms in Central America with quality broodstock.

### Significance

In Latin America, broodstock and seed supply have been identified as one of the major constraints to production increases. In the 2001 expert panel meeting organized by the PD/A CRSP, inadequate availability and quality of fry (and broodstock) were listed as a researchable priority. In southeastern Mexico, tilapia broodstock and fry quality are particularly poor. In the region, tilapia culture has been the principal aquacultural activity since the early 1970s. Unfortunately, the loss of the introduced lineages, the lack of effective genetic selection programs, and poor management decisions have created disappointment and uncertainty regarding tilapia culture. Thanks to CRSP funding (Work Plan 10), we have effectively obtained an F2 generation selected based on the combination of total length and condition factor. Comparisons made against the old broodstock group used in the government owned farm, a major seed producer in the region, showed that our broodstock produced an average 2.4 times more fry than the old line. Malformations are not present in the fingerlings produced, and survival and growth rates have been improved. Despite these encouraging results, the selection of at least an F4 generation is needed to ensure good quality broodstock. It has been reported that the selection of a fourth generation allows for the fixation of traits in a given tilapia population thus decreasing heritability values (Tave and Smitherman, 1980; Sanchez and Ponce, 1988; Tave, 1993, 1996).

The selective breeding program supported by the CRSP from 2001 to 2003 was initiated using 220 females and 110 males obtained from a batch of fish purchased from Egypt by the state government. A second line is currently being selected from wild animals. We have identified a stock of wild Nile tilapia in the Usumacinta River that shows several advantageous phenotypic traits (small head, small tail, large body, and uniform color). For the first year of work, we were able to combine the efforts of the CRSP project and another supported by the National Council for Science and Technology (CONACyT-Mexico). This action allowed us to work at the Mariano Matamoros Hatchery using 200, 1,000, and 2,000 m<sup>2</sup> ponds and to use fish first selected by Mario Fernández in 2000. To date, we have selected organisms from the second generation (F2) based on a combination of length and condition factor.

The establishment of good quality broodstock treatments, their distribution to local hatcheries, and the implementation of intensive masculinization programs are basic steps for sustainable aquaculture. These actions can significantly improve the production of high quality fingerlings and have a favorable impact on more than 5,000 subsistence farmers and medium-scale producers. Well-supported aquacultural practices can help secure good quality food products in the near future, especially in the proposed site of study where a large portion of the population is composed of extremely poor campesinos.

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In the current investigation, we would like to address some of the suggested lines of research identified in the Latin American and Caribbean CRSP expert panel meeting. Based on results of this study, we will propose an appropriate methodology for practical genetic improvement and we will characterize performance characteristics of available strains.

### **Quantified Anticipated Benefits**

Traditional genetic selection will generate broodstock capable of producing larger numbers of high quality fry than currently available. Fry and broodstock will be distributed to farmers.

### **Research Design**

#### **Experiment 1: Selective Breeding Program for Nile Tilapia in Tabasco Using Total Length and Condition Factor**

*Site:* Reproduction and progeny testing will be conducted at the Mariano Matamoros Hatchery, Teapa, Tabasco, Mexico. Groups of broodstock will also be kept at UJAT as a backup.

*Activities:* This study will be composed of three groups of broodstock for fry production and growth comparisons.

Group 1. Control group (old line from the Mariano Matamoros Farm)

Group 2. F2 Egypt line (CRSP project)

Group 3. F2 wild line (CRSP project)

Two hundred females and 66 males will be selected from the broodstock pools. Female selection will be based on the best (see below) total length measurements, and male selection will be performed using individuals with the best condition factor. Each selected broodstock group will be placed in a 200 m<sup>2</sup> concrete pond using a sex ratio of 3 females to 1 male for every 3 m<sup>2</sup>.

*Growth Performance:* Fry will be collected from the spawning ponds, and 28,000 fish from each group will be stocked in a 2,000 m<sup>2</sup> grow-out earthen pond. Fish will be fed five times a day providing a daily ration of 5% of the estimated biomass. Feeding charts will be constructed from samplings performed every two weeks. Sampling will be conducted after 60 days of grow-out and statistical comparisons will be made.

*Line Selection:* Ten percent of the fish from the growth performance phase of the Egypt and wild lines will be randomly collected. Fry will be divided in three groups using weight as the selection variable: 1) Fry which are 33% above the median value, 2) fry which are 33% around the median value, and 3) fry which are 33% below the median value. Group 1 will be reserved for follow-up studies, and group 2 will be used for line selection. All fish in group 3 will be discarded. From group 2 (of each line), 14,000 fish will be stocked in 1,000 m<sup>2</sup> earthen ponds (14 fish per m<sup>2</sup>) and grown-out for 2 months. At this time, 35% of the population (4,000 fish) that have the highest length will be selected and placed in 1,000 m<sup>2</sup> earthen ponds. After six months of growth, 35% (1,400) of the fish with the highest length will be selected to produce the F3 generation. Males will be selected based on highest condition factor, and females will be selected based on highest length.

*F4 Generation Selection:* The same protocol used to produce the F3 generation will be used to select and produce the F4 generation.

*Laboratory and Pond Facility:* The State government officials responsible for the Mariano Matamoros Hatchery have set aside four concrete ponds (200 m<sup>2</sup>) for spawning, four grow-out concrete ponds (1,000 m<sup>2</sup>), and four grow-out earthen ponds (2,000 m<sup>2</sup>) for UJAT's line selection investigation. If needed, more ponds can be used at the hatchery.

Universidad Juárez Autónoma de Tabasco (DACB): Two spawning concrete tanks (50 m<sup>2</sup>), 50 net cages (1 m<sup>3</sup>) for fry grow-out, three grow-out ponds (200 m<sup>2</sup>).

Universidad Juárez Autónoma de Tabasco (DACA): Two spawning concrete ponds (50 m<sup>2</sup>), two

grow-out ponds (50 m<sup>2</sup>), 20 net cages (1 m<sup>3</sup>) for grow-out.

The current tilapia line is composed of 600 females and 400 males.

*Stocking Rate for Spawning:* 3 females to 1 male, per 3 m<sup>2</sup>.

*Stocking Rate for Grow-out:* 14 fish per m<sup>2</sup> (first 2 months); 4 fish per m<sup>2</sup> (2 months); 20 fish per m<sup>2</sup>.

*Culture Period:* 9 months.

*Test Species:* Nile Tilapia (*Oreochromis niloticus*).

*Nutrient Inputs:* None.

*Water Management:* 10% water exchange per day.

*Sampling:* The following variables will be sampled:

- Initial weight and length
- Survival
- Final weight
- Final length

The following values will be estimated:

- Growth rate
- Condition factor
- Food conversion factor

*Statistical Methods and Hypotheses:* H<sub>0</sub>: Broodstock selected from the F2 and F3 generations will not have higher growth or fry production than those from the wild or the control groups. H<sub>A</sub>: Broodstock selected from the F2 and F3 generations will have higher growth or fry production than those from the wild or the control groups.

To determine differences in growth performance and fry production, ANOVA will be performed using weight, length, and mean number of fry per female as the dependent variables.

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## Nutrition and Nutrient Utilization in Native Peruvian Fishes

Fish Nutrition and Feed Technology 1 (11FNFR1)/Study/Peru

### Investigators

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Fred W. Chu	Graduate Student	Southern Illinois University at Carbondale

### Objectives

- 1) Assess the feasibility of utilizing native Amazonian plant products for small-scale sustainable aquaculture production of *Colossoma* and *Piaractus*.
- 2) Compare nutrient digestibility of endemic Peruvian plant products in paco (*Piaractus brachypomus*) with that of feedstuffs currently used in characid diets.

### Significance

A need exists to further evaluate the aquaculture potential of local and native species and to develop appropriate culture technologies. *Colossoma* spp., *Piaractus* spp., and their hybrids are important food fishes in the Amazon Basin. No uniform fish diets are available in the region (Cantelmo et al., 1986; Ferraz de Lima and Castagnolli, 1989). According to Van der Meer (1997), the ideal protein level has been determined to be approximately 43% for *C. macropomum*. Van der Meer also concluded excess soy in the diet tends to decrease palatability and growth rate. However, lower crude protein diets (~27%) have been successfully used at IIAP for many years (Alcantara; IIAP; personal communication), as well as in Brazil (Carneiro, 1981; Hernandez et al., 1992). The diets of wild *C. macropomum* are about 20 to 30% protein, with 75% of the protein being of plant origin (Araujo-Lima and Goulding, 1997). Fish diets greatly in excess of 30% crude protein would not likely be economically feasible in Amazonia.

Small-scale farmers often feed their fish domestic and wild fruits and vegetables, such as guavas, mangoes, potatoes, cabbages, pumpkins, bananas, rubber-tree seeds, manguba seeds, rice, corn, and manioc (Araujo-Lima and Goulding, 1997). Studies are also needed to assess the nutritional quality of the various plant products available and to develop an annual feeding regime based on the seasonal availability of the various fruits and vegetables. Araujo-Lima and Goulding (1997) have even suggested the development of "fish orchards" for feeding fruit-eating Amazonian fishes. Only in South America have fish communities evolved fruit- and seed-eating as a major part of the aquatic food chain (Araujo-Lima and Goulding, 1997). To some extent, these fish eat almost all fruit and seed species that fall into the water (Kubitzki and Ziburski, 1993). Adults feed to some extent on zooplankton, but fruits and seeds comprise the bulk of their diet. Although seeds seem to be preferred, large quantities of fleshy fruits are also consumed. Goulding (1980) and Kubitzki and Ziburski (1993) found that only occasionally are the seeds of these fleshy fruits masticated, but rather the fleshy fruit is swallowed whole and the seeds are defecated. Goulding (1980) has long proposed that the fruit-eating characins may play a double role as both seed predators and seed dispersal agents. Substantial data to support this hypothesis has been accrued in Work Plan 10 (Chu, dissertation in progress).

Culture techniques for native Peruvian fishes could be advanced considerably with new information on nutrient utilization in fishes fed diets with different compositions. Although some of the basic nutrient requirements are known for characids (St-Paul, 1985; Hernandez et al., 1995; Fernandes et al., 2001), there is no information on the availability of nutrients from feedstuffs of local origin. Even when cost and convenience of local feedstuffs are attractive, there is no advantage to using them in fish diets if the nutrients they contain are largely unavailable. The primary method of determining bioavailability of nutrients from individual feedstuffs is to determine the digestibility coefficients of different nutrients in individual feedstuffs during feeding trials. Digestibility coefficients for many of the feedstuffs used in current characid diets have been determined recently (Fernandes et al., in review). Comparative data from promising native feedstuffs would provide a nutritional basis for selecting low-cost accessible feedstuffs for use in characid diets in the Amazon region.

### **Quantified Anticipated Benefits**

The development of sustainable aquaculture of *Colossoma* and/or *Piaractus* will benefit many sectors throughout the Peruvian Amazon. Rural farmers will benefit by the addition of an alternative form of agriculture. Aquaculture production will require considerably less land than that needed for cattle ranching. Moreover, ponds can be used year after year whereas rain forest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons. Such lands, once abandoned, usually can no longer support normal jungle growth. Both rural and urban poor will benefit by the addition of a steady supply of high quality protein in the marketplace. Aquaculture of *Colossoma* and *Piaractus* should relieve some of the fishing pressure on these overharvested native species. The project will provide economic benefits to large-scale farmers by developing efficacious prepared diets and to small-scale farmers by developing a feeding regime using locally available plant products. The aquaculture of *Colossoma* and *Piaractus* should be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Peruvian Amazon. The combined results of these experiments will support CRSP goals of developing less expensive, more efficient feeds to improve culture techniques for indigenous Amazonian species. Increased availability of cultured fish should contribute to enhancement of human nutrition and health. In addition, increased aquaculture production should help relieve pressure on dwindling natural stocks of desirable foodfish species. Specifically, we will quantify the number of native plants showing promise as aquaculture feedstuffs.

### **Research Design and Activity Plan**

*Location of work:* This research will be conducted in Iquitos and Pucallpa, Peru (field work at IIAP).

#### *Methods:*

**Objective 1: Assess the feasibility of utilizing native Amazonian plant products for small-scale sustainable aquaculture production of *Colossoma* and *Piaractus*.**

Numerous wild fruits and plant products are reportedly utilized as fish feed in and around Iquitos (Table 1). To assess the feasibility of utilizing these and other plant products for small-scale sustainable aquaculture production of *Colossoma* and *Piaractus*, samples of fruits and plant products listed in Table 1 have been collected in Work Plan 10 as part of a dissertation by Fred Chu (Peruvian national). Proximate analysis of samples will be conducted at SIU Carbondale in cases where additional information is needed. Protein, amino acid, lipid, and fatty acid content of leaves, trunk, roots, flowers, and fruits, as appropriate, will be analyzed using standard techniques (Kjeldahl, Folch, spectrophotometry, and chromatography). In addition, data on the seasonal availability of the plants/plant products will be collected. Several seasonally-based feeding protocols will be developed based on the nutritional content and seasonal availability of the ingredients.

**Objective 2: Compare nutrient digestibility of endemic Peruvian plant products in paco (*Piaractus brachypterus*) with that of feedstuffs currently used in characid diets.**

Digestibility trials will be conducted in 110 L tanks in a flow-through system at UAPB using methods described in Reilly and Lochmann (2000). Four native feedstuffs will be tested using the indirect method and chromic oxide as a marker. Digestible energy, protein, lipid and dry matter digestibility coefficients will be determined for each feedstuff. The reference diet will be similar in composition to those used for characid fishes at IIAP currently. Water quality will be



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maintained by a flow rate of 750 ml per minute, and temperature will be held at 27±2°C.

Table 1. Proximal analysis of some fruits and other local plant products utilized to feed fish around Iquitos (food value per 100 g, modified from Morton, 1987).

Common Name	Scientific Name	Calories	Proteins (g)	Carbo-hydrates (g)	Lipids (g)	Fiber (g)	Ash (g)
Pijuayo	<i>Bactris gassipae</i>	196	2.6	41.7	4.4	1.0	N.D.
Guayaba	<i>Psidium guajaba</i>	36–50	0.9–1.0	9.5–10.0	0.1–0.5	2.8–5.5	0.4–0.7
Lady finger banana	<i>Musa paradisiaca</i>	110.7–156.3	0.8–1.6	25.5–36.8	0.1–0.8	0.3–0.4	0.6–1.4
Papaya	<i>Carica papaya</i>	23.1–25.8	0.1–0.3	6.2–6.8	0.1–1.0	0.5–1.3	0.3–0.7
Airambo	<i>Phytolaca rivinoides</i>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Mullaca	<i>Physalis angulata</i>	N.D.	0.05	N.D.	0.16	4.9	1.0
Cetico	<i>Cecropia</i> sp.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Míspero	<i>Achras sapota</i>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Renaco	<i>Ficus</i> sp.	80	1.2–1.3	17.1–20.3	0.1–0.3	1.2–2.2	0.48–0.85
Yucca	<i>Manihot esculenta</i>	135	1.0	32.4	0.2	1.0	0.9
Mishquipanga	<i>Renealmia alpina</i>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Picho huayo	<i>Siparuna guianensis</i>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cocona	<i>Solanum sessiliflorum</i>	N.D.	0.6	5.7	N.D.	0.4	N.D.
Cashew	<i>Anacardium occidentale</i>	N.D.	0.1–0.2	9.1–9.8	0.1–0.5	0.4–1.0	0.2–0.3
Caimito	<i>Chrysophyllum caimito</i>	67.2	72.0–2.33	14.7	N.D.	0.6–3.3	0.4–0.7
Anona	<i>Annona muricata</i>	53.1–61.3	1.0	14.6	1.0	0.8	0.60
Others:							
Rice meal powder	<i>Oryza sativa</i>	N.D.	6.2	36.0	5.2	28.9	15.7
Wheat bran	<i>Triticum aestivum</i>	N.D.	15.2	53.8	3.9	10.0	6.1

N.D. = No data available

### Regional and Global Integration

Research efforts being proposed are logical steps toward the continued development of sustainable aquaculture in the region as described in the regional plan. Research needs were identified with considerable input from in-country scientists and agency administrators. The research will benefit the entire region by providing pertinent information on feeding protocols. Data generated from this project should be applicable to most Amazon regions where characids are cultured. Data will also be useful for species with similar trophic habits.

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## Evaluation of Tilapia Aquaculture Best Practices in Central Luzon, the Philippines

Fish Nutrition and Feed Technology 2 (11FNFR2)/Activity/Philippines

### Investigators

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Christopher L. Brown	US Principal Investigator	Florida International University

### Objectives

This study aims to:

- 1) Establish a database on the range of current practices of farmers in a major tilapia-producing region in the Philippines, toward the establishment of best management strategies to guide the Philippines tilapia industry.
- 2) Update extension materials on the promotion of best feeding management practices.
- 3) Determine the optimal stocking size for tilapia grow-out through on-station experimental trials.

### Significance

A range of culture technologies is offered to tilapia farmers in the Philippines to increase fish production. Ironically, different research institutions impart different culture protocols presented during seminars or through extension materials, which causes confusion among tilapia farmers. Different levels of inputs are recommended to farmers, which result in varying yields and other production characteristics.

On-farm trials to test tilapia feeding strategies conducted under the sponsorship of the Pond Dynamics/Aquaculture Collaborative Research Support Program showed the economic benefit of reduced feeding (Brown et al., 2000; Bolivar et al., 2001). These feeding strategies will be continuously promoted through extension activities targeting the tilapia districts of Central Luzon.

### Anticipated Benefits

Extension materials will be updated to include more recent information on the best aquaculture practices for tilapia. Tilapia farming practices will be characterized according to locality or to some specific conditions (i.e., undrainable or drainable ponds) or management practice (supplemental feeding or complete feeding).

### Activity Plan

*Location of Work:* Survey and extension work will be carried out in the tilapia farming districts of Central Luzon. On-station studies will be done at the Freshwater Aquaculture Center, Central Luzon State University.

*Methods:* Defining the range of practices currently in use for the culture of tilapia will be the first step towards achieving the objectives of this investigation. Assessment of existing culture practices databases will be done. These databases will be obtained from current tilapia extension brochures, manuals, training curricula or modules, and even from broadcast media (television programs that provide information on agribusiness ventures such as tilapia farming). Existing databases will be analyzed on the following aspects:

- 1) Stocking sizes and densities
- 2) Fertilization schemes
- 3) Feeding strategies
- 4) Water quality management
- 5) Pond size or design

Actual farmer practices will be surveyed to determine what are the most common aquaculture

practices in the so called “tilapia region” in the Philippines. Survey questionnaires will be developed to cover information consistent with the gathered information from the database survey (i.e., stocking densities, initial stocking size, feeding and fertilization schemes, water management, and pond size). This activity will also be used to determine constraints that farmers face and how they make decisions related to their farming practices and the technologies that they adopt.

Survey methods will be designed in such a way that statistically useful data representative of regional trends in behavior, attitude, and decision-making priorities may be established. Standardized questions with quantifiable answers will be obtained. Rather than being asked “What do you do when dissolved oxygen levels appear low?” farmers will be asked something to this effect:

If dissolved oxygen levels appear to be low, I am likely to consider pumping compressed air into a pond: a) strongly agree b) agree c) no opinion d) disagree e) strongly disagree

Using this approach, along with a collection of demographic data included within the questionnaire, it will be possible to develop a statistically valid database of farmers’ practices, as well as to analyze correlative patterns among the answers. This will serve as a baseline for possible shifts in farmers’ practices, as well as a guide in the effective dissemination of new technology. The questionnaire will be designed for analyses of results using ANOVA and appropriate multiple comparison tests (SNK or Duncan’s Multiple Range Tests). We expect, for example, to generate a quantitative profile of attitudes about:

- The use of antibiotics and other medications.
- Confidence in various sources of fry or feeds.
- Relative willingness to adopt new measures from extension agents, competing farmers, or from other sources.
- Desire to intensify production.
- Willingness to assume additional risks or up-front costs to increase production.
- Preventative and remedial measures.
- Concern for environmental quality.
- The relatedness of patterns among the various trends.

We believe this will be useful because it will illustrate how farmers in Central Luzon make decisions about tilapia farming technology, which in turn may allow us to take the most effective approach in the dissemination of technical information to them.

### *On-Station Research:*

#### **Effect of Stocking Size on Yield and Survival of Nile Tilapia On-Grown in Ponds**

The stocking size of tilapia for grow-out pond production has been dictated by the common size of fingerlings available from tilapia hatcheries. For instance, since size 24 is the most common and readily available fingerling size, farmers tend to stock at this size directly into grow-out ponds. In the past, farmers obtain fingerling size as big as 20 g from hatcheries to stock in their grow-out ponds. This requires a two-staged production system where fry from hatcheries are transferred to nursery ponds and then stocked into grow-out ponds when they attain the weight of about 20 g. Bolivar and Lanuza (in preparation) studied the effect of different stocking sizes on the growth, yield, and survival of tilapia on-grown in ponds. There were no significant differences on the growth, yield, and survival of Nile tilapia stocked at different sizes after 90 days of culture. However, this may be due to the small size differences of tilapia fingerlings used at stocking.

Bigger-size fingerlings are thought to survive better, grow faster, and reach market size sooner. Production characteristics of pond culture with a nursery phase to produce stocker-size fingerlings has not been evaluated.

The objective of this study is to determine the effect of fingerling size on yield, growth, and size variability at harvest in order to examine the production characteristics of Nile tilapia cultured in grow-out ponds. Two culture phases will be implemented: that of a nursery phase and the grow-

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out phase. For the grow-out phase, size 24 fingerlings of Nile tilapia will be grown in nursery hapas until they attain the weight of about 20 g. A suitable stocking density of tilapia fingerlings in the nursery hapas to attain 20 g will be established.

Fingerlings will be fed to satiation with fry mash. To ensure size uniformity before stocking into the grow-out ponds, the fingerlings will be size graded. For the grow-out phase, graded tilapia fingerlings will be stocked in ten 0.05 ha earthen ponds at a stocking density of 5 fish per m<sup>2</sup>. Two stocking sizes will be designated as treatment: I – direct stocking at size 24; and II – stocking at 20 g size tilapia fingerlings. Each treatment will be replicated five times. The fish will be fed to reduced satiation (67%) with commercial tilapia pellets beginning at 60 days after stocking. Monthly sampling of fish will be done to monitor growth. The ponds will be harvested by total draining after 90 days of culture and relevant data (fish size, weight, and number) will be collected.

*Statistical Analysis:* Two sets of analyses will be carried out. First, the effect of initial stocking size on the production characteristics listed above will be determined using the paired student's T-test for comparison. In addition, a cost analysis will be developed in the nursery phase to determine the costs of producing stocker-size fingerlings, in the grow-out phase to compare the cost of production in each treatment, and to calculate the net production cost for each of the two approaches. These costs will be deducted from calculated crop values, as in our previous analyses of feeding methods, to obtain a reasonable indication of profitability.

### Regional Integration

Central Luzon State University has a vigorous program of aquaculture extension, and this investigation is designed not only to address and document area farmers concerns, but also to update current extension materials. The atmosphere at the College of Fisheries campus at CLSU is cosmopolitan; CLSU draws students and graduate students from an international base and increasingly presents and publishes research, including PD/A CRSP research, in international journals. For these reasons, we are confident that technical progress made in the course of this investigation will be quickly and thoroughly disseminated.

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## Use of Phytochemicals as an Environmentally-Friendly Method to Sex-Reverse Nile Tilapia

Fish Nutrition and Feed Technology 3 (11FNFR3)/Experiment/Mexico

### Investigators

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Konrad Dabrowski	US Principal Investigator	The Ohio State University

### Objective

Evaluation of potential action of purified phytochemicals on sex differentiation of tilapia by dietary and immersion administration.

### Introduction

In tilapia aquaculture, all-male populations are desirable because males demonstrate superior growth characteristics when compared to females (Mbahinzireki et al., 2001). Moreover, culture of mono-sex populations prevents reproduction and results in uniformity of fish size. One method commonly used to induce sex reversal is the oral administration of 17 $\alpha$ -methyltestosterone (MT) during the period of gonadal differentiation of the fish (Green et al., 1997; Abucay and Mair, 1997; Gale et al., 1999). However, the efficacy of MT is dependent on various factors such as dose, timing and duration of treatment, and mode of administration (Mirza and Shelton, 1988).

One problem associated with the use of MT is that, at high doses or prolonged treatment, MT induces gonadal intersexuality and/or paradoxical feminization (Goudie et al., 1983; Solar et al., 1984; van den Hurk et al., 1989; Blasquez et al., 1995; Rincharde et al., 1999; Papoulias et al., 2000). The second reason for restricted use of MT is its environmental hazard. Therefore, alternative methods to produce all-male tilapia populations are needed and are among the priorities of the PD/A CRSP. Phytochemicals, naturally found in numerous plants, exhibit an endocrine disruptive activity since they interfere with various enzymatic reactions either in steroid metabolism (aromatization) or in the mechanism of action (competitive receptor binding) of the steroids (Pelissero et al., 1996; Geahlen et al., 1989). Therefore, we intend in the Eleventh Work Plan to continue the isolation and development of a synthetic, steroid-free method for producing all-male populations of tilapia using either a suite of active compounds (plant extracts) or specific purified phytochemicals. The work intends to administer purified chemicals both by dietary and immersion exposure and use extracts from plants that are considered to have some effect on the hormonal disruption. Therefore, both mechanisms, enzymatic inhibition or receptor competition (as agonist or antagonist), will be considered in our approach. In this approach natural high concentrations of phytochemicals in respect to tilapia diets and possible synergistic effect of several phytochemicals should be emphasized. We hypothesize that feeding fish with diets containing these natural substances will affect the sex ratio of the tilapia populations. Recent scientific information and the preliminary results from the experiments carried out during the Tenth Work Plan show the necessity to expand the number of phytochemicals to be evaluated, along with the method of administration, and the evaluation of natural sources of these chemical compounds (plant extracts) for dietary inclusion.

### Significance

In tilapia aquaculture, all-male populations are desirable because males grow faster than females. Moreover, culture of mono-sex populations prevents reproduction and results in uniformity of fish size. The synthetic steroid MT is a derivative of a male-specific hormone commonly used to masculinize

tilapia juveniles (Green et al., 1997; Abucay and Mair, 1997; Gale et al., 1999). The effect of MT is dependent on various factors such as dose, timing and duration of treatment, and mode of administration (Mirza and Shelton, 1988). MT is known to be associated with problems when administered at high doses or over a prolonged time period. MT induces gonadal intersexuality and paradoxical feminization (Goudie et al., 1983; Solar et al., 1984; van den Hurk et al., 1989; Blasquez et al., 1995; Rincharde et al., 1999; Papoulias et al., 2000). Piferrer and Donaldson (1989) suggested that paradoxical feminization might be due more to aromatization than to inhibition of in vivo synthesis of androgens. However, MT release and environmental concerns are a major reason behind a search for alternative, environment-friendly chemicals.

One approach may involve the use of pure chemical agents (phytochemicals). These substances may interfere in hormonal regulation at the following levels: steroid receptor-mediated actions and modulation of endogenous steroid production and bioavailability (Mäkelä et al., 1999). Isoflavonoids such as genistein and daidzein act as estrogen agonists via estrogen receptors in cultured cells and also manifest estrogen-like effects in the female reproductive system (Miksicek, 1995; Santell et al., 1997). Flavonoids, such as chrysin, are natural aromatase inhibitors (Chen et al., 1997) and may be used to boost low levels of testosterone in aging males. A plant sterol, spironolactone, has a high androgenic antagonist receptor activity. However, there is evidence that it can cause paradoxical masculinization at low concentrations in mosquitofish, *Gambusia affinis* (Howell et al., 1994). Phenolic acids such as caffeic acid act at Leydig cell hormone receptors in rat testicles, blocking the LH-stimulated testosterone production (Mele et al., 1997). In the Eleventh Work Plan, we intend to continue the evaluation of the potential action of different phytochemicals. Considering that only few references exist on the aromatase inhibition coefficient of isoflavonoids and phenolic acids in fish in vitro (Bennetau-Pelissero et al., 2001; Joshi et al., 2002), we propose to examine the effect of chrysin, daidzein, caffeic acid, and spironolactone on sex differentiation of tilapia. Our laboratory is able to isolate and quantify those phytochemicals in fish tissues using high-performance liquid chromatography (HPLC) methods.

Besides the use of purified phytochemicals, we propose the second approach—the use of plant extracts with known characteristics of phytochemicals of interest. There is evidence that chemicals contained in plants could provide a useful source of masculinizing agents (Lohiya et al., 1999). Consequently, the detailed evaluation of such information is proposed in this Eleventh Work Plan. Several reports provide evidence that gastric intubation of aqueous extracts of *Hibiscus macranthus* and *Basella alba* into rats had anabolizing and virilizing effects (Moundipa et al., 1999) and may be used to boost low levels of testosterone in aging males. Maca (*Lepidium meyenii*) tuber meal has long been used as a remedy for the treatment of human male's infertility in Peruvian rural communities (Quiros et al., 1996; Cicero et al., 2001; Gonzales et al., 2001a). Recently, oral administration of maca extracts was reported to enhance sexual behaviors in male mice and to decrease erectile dysfunction in male rats (Zheng et al., 2000). Cicero et al. (2001) also reported that the oral administration of pulverized maca root improved sexual performance of male rats. In rats, aqueous extracts of maca roots increased weights of testis and epididymis and activated spermatogenesis after 14 days of oral administration (Gonzales et al., 2001b). Testosterone is a hormone well-known for maintaining and/or activating spermatogenesis. Therefore, we hypothesize that maca and other plant extracts would have androgenic effects that would potentially convert female tilapia to males. Our laboratory in Columbus has extensive experience on using maca meal as a feed intake enhancer in salmonid alevins (Lee and Dabrowski, 2002).

### **Anticipated Benefits**

The use of phytochemicals as an alternative method to produce mono-sex populations of tilapia will address human and environmental safety issues. Fish offered to the consumer will not be treated with MT, and producers may have an alternative method for producing all-male populations of tilapia based on natural products, which do not require FDA approval. The low cost of using phytochemicals or plant extracts should also be an attractive alternative to producers. Moreover, several phytochemicals have proven antioxidant activity (prevent oxidation of dietary lipids) and enhance immune resistance of fish.



## Research Design

*Location of Work:* The feeding experiments will be performed both at the Laboratory of Aquaculture, Universidad Juárez Autónoma de Tabasco, Mexico, and at the School of Natural Resources, The Ohio State University, Columbus, Ohio. The experimental diets as well as radioimmunoassay and HPLC analysis will be carried out at OhSU. The Aquaculture laboratory at OhSU has extensive experience in nutrition, reproduction, and sex reversal in tilapia (Mbahinzireki et al., 2001; Rinchard et al., 2002). We continue to work on phytochemicals in fish diets and their metabolism (Lee et al., 2001; Dabrowski et al., 2001). Over the years, several experiments in our laboratory involved the use of MT and monitoring concentrations of this chemical in fish tissues (Rinchard et al., 1999).

## Methods:

Evaluation of Potential Action of Purified Phytochemicals on Sex Differentiation of Tilapia by Dietary and Immersion Administration

The experiments will be conducted simultaneously on first feeding genetically all-female or all-male tilapia, *Oreochromis niloticus*, in the same conditions using the same batch of fish for both administration modes. For both experiments, fish will be randomly distributed into 24 aquaria at a density of 150 fish per aquarium with three replicates per treatment. Initial data on weight and proximal composition will be gathered from an initial sample of 100 fish. Fish will be fed on a restricted ration up to 90% satiation for 8 weeks. Semi-purified diets will be formulated based on our previous experience (Lee et al., 2001). Semi-purified diets (casein-gelatin-dextran based) will be used to reduce the presence of natural steroids in the ingredients of plant or animal origin used for practical diet formulations (Feist and Shreck, 1990). In order to determine the effect of phytochemicals in sex differentiation, seven casein-gelatin based diets will be prepared. Diet 1 (negative control) will be free of phytochemicals. Diets 2 and 3 (positive controls) will contain either MT at a dose of 40 mg kg<sup>-1</sup> (Abucay and Mair, 1997) or ATD (1,4,6-androstratiene-3-17dione aromatase inhibitor) at an initial dose of 150 mg kg<sup>-1</sup> (Guigen et al., 1999). Diets 4 to 8 will contain chrysin, daidzein, caffeic acid, and spironolactone at a dose of 500 mg kg<sup>-1</sup>. These amounts correspond to the levels commonly found in seed meals of plants (Benneteau-Pelissero et al., 2001; Dabrowski and Lee, 2001). Subsequently, different concentrations of the phytochemicals will be tested. For the immersion treatments, the phytochemicals mentioned above will be dissolved in two different vehicles, ethanol or dimethyl sulfoxide (DMSO). The LD50 will be determined for each phytochemical prior to experimental exposure. No-effect dose will then be chosen. Groups of 150 fish will be exposed (three groups per treatment) during an optimized time (week one to four) of sex differentiation (W. Contreras-Sánchez, personal communication). In both experiments water quality will be monitored throughout the feeding experiment. Temperature and dissolved oxygen will be determined on a daily basis with biweekly measurements of total ammonia-nitrogen and pH. At the end of the feeding trial, growth performance will be evaluated in terms of individual body weight, survival (%), specific growth rate (%), and weight gain (%). Fish from each dietary treatment will be sampled for proximal body analysis (moisture, protein, lipid, and ash) and phytochemical analysis (Dabrowski et al., 2001). Gonadal development and differentiation will be determined by histological analysis at weeks 4 and 6. Sex ratio will be evaluated by microscopic analysis of gonadal squashes (Guerrero and Shelton, 1974; Guigen et al., 1999) at the end of the experiment.

*Statistical Analysis:* Analyses will be performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Data on growth performance and survival will be subjected to one-way analysis of variance (ANOVA) followed by a comparison of means using Scheffe's F test (Dagnelie, 1975).

The Chi-square test will be used to determine alterations in sex ratios. Normality and homogeneity of variance tests will be performed on raw data. Sample distributions violating assumptions will be log-transformed before analysis. Data, expressed as percentages, will be arc sine-transformed before analysis. All differences will be regarded as significant at  $P < 0.05$ .

## Schedule

June–August 2003: Procurement by OhSU, Columbus, Ohio, and UJAT, Tabasco, Mexico of genetically all-female and all-male tilapia.

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July–August 2003: Preparation of diets for dietary administration and determination of LD50 for the different phytochemicals to select “no-effect” dose.

Diets:

- Diet 1: Negative control, free of phytochemicals
- Diet 2: 40 mg kg<sup>-1</sup> methyltestosterone
- Diet 3: 150 µg kg<sup>-1</sup> ATD (1,4,6-androstratiene-3-17-dione)
- Diet 4: Diet containing 500 mg kg<sup>-1</sup> chrysin
- Diet 5: Diet containing 500 mg kg<sup>-1</sup> daidzein
- Diet 6: Diet containing 500 mg kg<sup>-1</sup> caffeic acid
- Diet 7: Diet containing 500 mg kg<sup>-1</sup> spironolactone

For immersion:

- Treatment 1: Ethanol only
- Treatment 2: 400 mg kg<sup>-1</sup> methyltestosterone
- Treatment 3: 400 mg kg<sup>-1</sup> ATD
- Treatment 4: Chrysin
- Treatment 5: Daidzein
- Treatment 6: Caffeic Acid
- Treatment 7: Spironolactone

Note that prior to immersion experiments, LD50 studies will be carried out.

September–November 2003: Carry out feeding and immersion experiments.

Parameters: Individual body weight, survival %, specific growth rate, weight gain, proximal analysis, gonadal development, sexual differentiation by histology at weeks 4–6, sex ratios at end of experiment.

December 2003: Phytochemical and MT analysis in feeds, water, and fish tissues.

January–March 2004: Summarization of results and statistical analyses.

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## **Insulin-like Growth Factor-I as a Growth Indicator in Tilapia**

Fish Nutrition and Feed Technology 4 (11FNFR4)/Experiment/Philippines

### **Investigators**

Remedios Bolivar	HC Principal Investigator	Central Luzon State University, Philippines
Christopher L. Brown	US Principal Investigator	Florida International University
Russell Borski	US Co-Principal Investigator	North Carolina State University

### **Objectives**

This investigation builds on progress made during Work Plan 10, which at the time of submission is early in the second year of funding. A highlight of that project (10RCR3) has been the successful cloning of insulin-like growth factor-I (IGF-I) in the tilapia, *Oreochromis niloticus*, which enables us to begin examining the relationship of this compound to the growth process and to test the feasibility of its use as an instantaneous growth indicator. In the course of Work Plan 10, we have completed preliminary experiments designed to elucidate relationships of this regulatory compound with growth rate in tilapia. Our overall goal for Work Plan 11 will be to use molecular probes to quantify IGF-I and establish in a definitive way its relationship to somatic growth.

The specific objectives include:

- 1) Experimentally determine the relationship of plasma and tissue concentrations of IGF-I as it relates to growth rate in the tilapia, *Oreochromis niloticus*.
- 2) Examine ontogenetic patterns of tissue IGF-I expression during the initial onset of growth in juveniles.

### **Significance**

Over the years, the Pond Dynamics/Aquaculture CRSP has investigated innumerable combinations of biotic and abiotic factors as they impact the growth of cultured tilapia, *Oreochromis niloticus*. The variation of feeding, fertilizing, stocking density, genetics, and other parameters is known to impact feed conversion efficiency and consequently growth—endpoints which are conventionally determined at the time of harvest.

Considerable time and expense are required to ascertain gross measures of weight and length gain on a mass-culture scale and in the various regions that absorb the technical products of these investigations. Considering the extensiveness of this process, coupled with the multitude of factors regulating growth, an instantaneous indicator or predictor of growth would be a valuable tool in the hands of experimental tilapia culturists. During Work Plan 10 we began to address this issue as the subject of a doctoral research project now underway by Emmanuel Vera Cruz. Mr. Vera Cruz is a faculty member in good standing at Central Luzon State University and a doctoral candidate at Florida International University. His research is based on the premise that the need for a means of rapid assessment of growth can be overcome, in part, by development of functional genomic and blood markers to instantaneously assess growth responses of tilapia to any culture parameter. Subject to sufficient validations now underway, this would preclude long grow-out trials inherent with traditional studies, leading to accelerated diagnostics for superior growth performance.

A potentially good candidate protein and gene is that for insulin-like growth factor-I (IGF-I). IGF-I is the definitive mediator of growth in all vertebrates where it acts to induce cellular proliferation and growth (hyperplasia and hypertrophy). The growth-promoting actions of growth hormone are mediated through induction of IGF-I (Siharath and Bern, 1993; Duan, 1997). Natural or exogenous growth hormone enters the circulation and induces release of IGF-I from the liver, the primary source of circulating IGF-I, which acts systemically to increase growth (Duan, 1997; Chen et al., 2000). Circulating IGF-I also prevents excessively high production of growth hormone in mammals, a phenomenon that



also applies to fish including the tilapia (Fruchtman et al., 2000; Fruchtman et al., 2002). Growth hormone and other factors (e.g., steroids, nutritional components) also induce production of IGF-I by peripheral tissues, such as muscle and cartilage, where it acts locally to induce cell hyperplasia and hypertrophy, cartilage matrix deposition, protein synthesis, and overall tissue growth (Negatu and Meier, 1995; Jones and Clemmons, 1995; Duan, 1997). Deletion of IGF-I by null mutations in gene “knockout” experiments leads to severe growth retardation and developmental defects in brain, bone, and muscle (LeRoith et al., 2001).

Because it is such a critical regulator of animal growth, it is not surprising that tissue levels or circulating concentrations of IGF-I correlate well to anabolic growth status (Borski et al. 1994, 1996; Perez-Sanchez et al., 1994; Nicoll et al., 1998; Beckman et al., 1998). Dr. Borski’s research on hybrid striped bass revealed a clear relationship between growth rate and blood IGF-I levels. His group found that systemic IGF-I concentrations are elevated in animals undergoing accelerated (compensatory) growth as compared to control animals (in preparation). These results verify that not only do rises in IGF-I levels accompany increased growth, but that the degree of growth enhancement corresponds to the magnitude of increase in IGF-I concentrations.

### Anticipated Benefits

The primary benefit of the proposed work will be the establishment of a means of quantifying growth in tilapia that does not require costly and time-consuming grow-out. With such a method, it should be possible to optimize culture parameters in a far more rapid and focused way than is possible with the present technology, reducing the time and cost involved in improving practical food production methods. The potential number of direct beneficiaries (tilapia farmers in the Philippines) runs well into the thousands and indirect beneficiaries of a more stable and sustainable protein supply could be more numerous.

Coupled with the development and validation of tools, we should add that this technology will be transferred readily and directly to the tilapia-growing region of the Philippines, since the doctoral student carrying out these studies will return to his post as a Central Luzon State University faculty member upon completion of his doctorate. Presumably the rapid assessment of growth using IGF-I assay or probe methods will be among services available at the Freshwater Aquaculture Center and hence on hand for testing of instantaneous growth responses in the continued development of culture parameters for the tilapia and possibly other species. Technical backup will also be provided from US collaborators as needed.

### Research Design

During the current funding cycle (Work Plan 10), we worked with Dr. Borski to successfully clone a portion of the Nile tilapia (*O. niloticus*) IGF-I gene using primers generated from striped bass and *O. mossambicus* sequences (Chen et al., 1998; Fruchtman, 2001). We have generated a 330 base pair cDNA sequence corresponding to the B-D domain of the IGF-I gene and inserted it in an appropriate vector that allows generation of a DNA or RNA probe that specifically complements *O. niloticus* IGF-I mRNA(s). With this gene in a suitable vector, we may generate various forms of molecular probes that can be used in the detection of IGF-I and finalize the analysis of samples from an experiment designed to test the use of this compound as an indicator of growth.

With the establishment of a highly specific and sensitive means of detecting IGF-I mRNA levels in liver and muscle, the proposed research will lead us into examinations of muscle and liver IGF-I mRNA and plasma IGF that might serve as good indices of growth in tilapia, as it appears to in salmonids and temperate basses. This type of growth biomarker assay will be useful for rapidly assessing optimal conditions that best promote growth of pond-cultured Nile tilapia. Results from these studies should also advance our understanding of the fundamental mechanisms mediating growth in tilapia.

### Preface: Assay Validation (Being completed as part of Work Plan 10)

The RNase protection assay is a quantitative method in which a radiolabeled probe is coincubated with a tissue sample, and specific hybridization occurs. The RNase enzyme is added and only the



specific hybrids resist degradation. This will allow us to detect IGF-I in a sensitive and highly specific manner. Validation of the assay will involve the addition of known amounts of IGF probe to establish the amount needed to saturate all IGF mRNA within samples (saturation), determination of the specificity of the probe by examining the size of the “protected” mRNA (should be the same as that of the cloned fragment), and detection in tissues collected from animals with known growth status (biological confirmation test). These studies will be done at North Carolina State University in the laboratory of Dr. Borski with the active participation of Mr. Vera Cruz. Plasma IGF-I will be measured using a Universal fish IGF-I radioimmunoassay (GroPep Ltd.) already validated for tilapia.

## Part 1: Growth Studies

Once validated, the RNase protection assay can be used to study in detail the relationship of IGF to growth and the accuracy and predictive power of IGF-I measurement as an indicator of growth rate. A series of experiments will address these questions, all based on the null hypothesis that the regulatory compound, IGF-I, has no relationship to the pattern of growth in the tilapia.

Laboratory trials (Florida International University) will have groups of tilapia housed in aquaria on different feeding regimes based on our prior experimentation with sub-satiation diets. These will include 100% satiation, 75% satiation, and 50% satiation. It is anticipated that at least the last of these three groups will grow at a slower rate; body mass will be determined weekly over the course of four months (a fairly typical grow-out schedule for tilapia) and samples will be collected for quantification of IGF-I using the RNase protection assay. The correlation of growth in terms of body mass and measured IGF-I levels will be subjected to statistical analysis using ANOVA.

Because we will sample animals weekly, our results will be displayed with time as the independent variable and expression of IGF-I and body mass as dual dependent variables (using two y axes). This will serve to illustrate not only the timing of perceptible increases in IGF-I in growing fish, which in theory will precede actual increases in body mass, but also the general pattern of correlation of IGF-I with growth rate. Taken together, these results should serve as a biological validation of the efficacy of IGF-I detection by the RNase protection assay as an indicator of growth rate.

## Part 2: Ontogenetic Study

Many feel that at the time of the onset of growth, patterns of growth are established that persist throughout adulthood, and hence this transitional phase may play a determining role in agricultural fish production. The availability of the tilapia IGF-I sequence will allow us to generate (based on the sequence) and test additional probes capable of detecting this regulatory compound in tilapia tissues. In addition to the sort of RNA probes used in the protection assay, we will generate fluorescent-labeled oligonucleotides that react specifically with the target compound, IGF-I RNA. Fluorescence will be sought in tilapia specimens during the transition from larval stage, which is characterized by extensive development but relatively little growth, into the juvenile stage, which is growth-intensive. Both sections through whole larvae and the in situ approach using entire larvae will be used in the confocal microscopy center at the Florida International University Biscayne Bay Campus. This will not only indicate when and under what physical conditions (e.g., temperature, diet) the growth mechanism is initially activated, but in what specific organs or tissues. Our attention will focus on expression of IGF-I as a function of time, temperature, photoperiod, feeding, and other dietary variables in liver and muscle throughout the transition from development to grow-out.

Much attention has focused on selective breeding for rapid growth with relatively little attention to the physiological factors that determine growth rate or feed utilization efficiency. An understanding of the dynamics of the onset of growth as quantified by IGF-I detection will allow us to examine nutritional, environmental, and even social variables and to optimize hatchery protocols.

### Regional and Global Integration

This project will develop and test an innovative technique for determining growth rates that does not require long grow-out. Initially, it will be developed at North Carolina State University and should be available for transfer to the Freshwater Aquaculture Center at Central Luzon State University by the time Mr. Vera Cruz completes his doctorate (around 2005) and returns to CLSU. This is a technical development that can and probably will be applicable for the numerous and multifaceted studies of tilapia production technology at CLSU, many of which have enhanced growth as a focus. We believe that a successful launch of an instantaneous growth assessment tool of this sort, followed by its transfer to a prominent regional aquaculture technology center such as CLSU is the best and most efficient way of making the method available throughout southeast Asia.

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## **ELEVENTH WORK PLAN**

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## Synopsis for Project under Development

### Africa Project: Watershed Management

#### Collaborating Institutions

Lead Kenya Institution: Moi University, Kenya

Other Kenya collaborators: Department of Fisheries, Kenya

Kenya Wildlife Service, Kenya

Lead US Institution: TBA

Other US collaborators: TBA

#### Objectives

The overall goal of the proposed project is to apply a multidisciplinary approach to develop and demonstrate improved and integrated sustainable management watershed resources through stakeholder participation at the watershed scale. The vision of the project is to reverse the decline of water quantity and quality over a two to five-year period. The first year will be devoted to preliminary implementation of the following broad objectives:

- 1) To develop data sets that are historical, technical and stakeholder-centered;
- 2) To identify and consult with stakeholders and incorporate their ideas in long term intervention;
- 3) To review and document previous management plans and interventions, if any, of various portions of the rivers near the Njoro watershed and determine what elements of these plans have been implemented and how effectively these activities have been sustained;
- 4) To facilitate communities as partners in the data collection, design, planning, implementation, and evaluation of a strategy to rehabilitate the watershed; and
- 5) To develop the capacity of Moi University and the Kenya Department of Fisheries to become a regional center in watershed resource management.

#### Significance

Over the years, degradation of water resources in terms of quantity and quality within and around the watersheds near Njoro have occurred due to poor watershed management. This has resulted in serious degradation of the ecological integrity and hydrologic processes within the watershed. This is shown by the loss of biodiversity and habitats. Consequently, the trend has resulted in declining livelihoods of the inhabitants and their livestock. These factors have contributed to overall poverty in the area. With this background, there is a need to develop strategies and mechanisms to rehabilitate the watersheds in the region. The proposed project will embark on a multidisciplinary approach to develop and demonstrate improved and integrated sustainable management of watershed resources at a watershed scale. Overall, the proposed project is a first step in creating a sustainable interdisciplinary watershed rehabilitation model through technical social and policy interventions that would enable Moi University and the Kenya Department of Fisheries to become regional centers in watershed resource management.

The current situation in and around watersheds near Njoro are not sustainable. The current *laissez faire* approach neither protects the environment nor involves or protects the public. There has been no practical research or outreach, which has attempted to link water, environmental sanitation, and hygiene with development, public health, or poverty alleviation in the region. Urban and rural people alike will suffer as a result of the shortsightedness that allows such unbridled resource exploitation to continue. A new approach for catchment-scale water and resource management is clearly needed.

#### Research Design

The Project consists of six components namely: 1. Stakeholder Involvement; 2. Ecology; 3. Socio-economic; 4. Watershed Hydrology; 5. Capacity Building; and 6. Gender Activities.

The components will be integrated through multidisciplinary approaches to investigate watershed conditions. The project adopts methods, which lead to stakeholder involvement and capacity building,

## ELEVENTH WORK PLAN

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through hydrologic, ecological and sociological surveys.

### Stakeholder Involvement Component

Watershed stakeholders include all persons, agencies, or institutions living in or, participating in the use and/or management of natural resources within the watershed. The primary objective of involving stakeholders is to engage them in planning, implementation, and management processes necessary to improve watershed resources. Sustaining stakeholder involvement ensures the sustainability of beneficial practices and dissemination of essential information to all stakeholders and embodies the concept of ownership of their resources.

This component will include the development of a model of stakeholder involvement to actively engage stakeholders in the process of problem/needs/opportunity assessment. This will foster effective communication between stakeholders, policy makers, and scientists to make each aware of the others concerns and knowledge base(s) is essential to engaging stakeholders in the process of active and sustainable involvement. This in turn will provide stakeholders with the necessary knowledge to participate effectively in decision-making, planning, and rehabilitation activities.

### Ecology Component

The primary goal of the watershed ecology research component is to establish the ecological health and potential of aquatic and terrestrial ecosystems. The health potential of the watershed will be inferred by integrating current and historical conditions at a sampling site of similar, unimpaired reference. This acts as a benchmark against which data from watershed health surveys will be compared, to determine the existence of any impairment at the sampled sites. Impairments are defined as deviations from the normal expected natural site conditions. The magnitude of the divergence from the expected site conditions represents the severity of impairment. Developing a benchmark of watershed health potential will be an initial step in setting general watershed rehabilitation goals.

Protocols for monitoring biophysical characteristics of the landscape conditions at sampling sites will be developed throughout the watershed in order to capture the spatial distribution of landscape conditions as a function of biophysical and anthropogenic activities.

The collected data will serve as a basis for landscape assessment and formulation of prototype indices, biological monitoring and ecological decision support model for use by resource managers, researchers and other stakeholders. An interactive approach will be used to achieve this objective. The process will enhance information dissemination to stakeholders and augment the utilization of the data.

### Socioeconomic Component

The pressing socioeconomic demands of an increasing population in watersheds tend to dictate the levels of resource use. Governments in many developing countries are unable to establish relevant policies to regulate use while maintaining the condition of the base resources. Consequently, the resource users have continued to exert pressure on the resources to provide for their basic needs at the detriment of the watersheds. Utilization of such resources often is unsustainable leading to the general impoverishment of the people and the environment.

Among the various problems in the watershed, is the vicious cycle of poverty arising from intensified cultivation of riparian ecosystems as well as steep slopes at high altitudes without proper conservation structures. The intensification of socioeconomic activities in the watershed is suspected to be responsible for the loss of watershed integrity and observable loss of water quality and quantity.

In view of the importance of human activities in the watersheds, there is need to assess the socioeconomic status of resource users in order to develop data sets that would be used for project monitoring and evaluation.

### Watershed Hydrology Component

The present hydrological conditions of the watersheds are as a result of many processes and activities



both anthropogenic and natural. Poor hydrologic conditions of watersheds are known to contribute greatly to the deterioration of watersheds particularly with respect to the water quality and quantity. It is necessary to understand the interrelationship of these processes in order to suggest interventions that will lead to the arresting of these conditions and possibly reversing them.

Because of the above facts, it is important to understand the various physical aspects of the watershed and their contribution to the overall hydrologic status i.e., its water quality and quantity conditions. By carrying out an in-depth inventory/characterization of the hydrological condition, it is possible to identify pressure points, which ultimately form the entry avenues for the interventions. The rehabilitation as a goal for most elements of the watershed presupposes knowledge of the current and reference conditions of both functioning and nonfunctioning watershed processes. Thus, both qualitative and quantitative data will be a prerequisite for the watershed rehabilitation.

The ultimate goal for the watershed hydrology component will, therefore, be to carry out an in-depth characterization of the physical characteristics of the watershed and to integrate the other ecological as well as the anthropogenic characteristics to effectively rehabilitate the watershed.

### Capacity Building

Capacity building will prepare the assessment team members to approach the formulation of the research proposal as a single team to accomplish the desired goal of improved water quantity and quality. The assessment team members are expected to attain basic skills in PRA, system analyses, watershed management and Geographical Information Systems (GIS) in order to form a highly interactive and integrated approach to identifying goals and problems of the proposed research project. Training in project and budget management is designed to ensure the long-term success of these research activities.

### Gender Activities

Women are centrally responsible for domestic water supplies, family health and hygiene, and carry out important roles in both farm and non-farm household income production activities that have implications for the sustainability of watershed resources. Furthermore, a larger portion of poorer households in rural and urban areas tends to be women-headed. For this reason, gender analysis will be included in the participatory rural appraisal methods to be used during the assessment phase with communities in laying the foundation for stakeholder involvement in managing the watershed. The inclusion of women representatives in the planned exposure visits, tiered workshops, and stakeholder trainings will also be key to assuring that diverse stakeholder interests, perspectives, and impacts, particularly for poor households, are represented in developing interventions and management plans for the watershed.

### Synopsis for Project under Development

#### Latin America and the Caribbean: Human Welfare, Health, and Nutrition Strengthening Aquaculture Extension Networks and Improving Skills as the Vehicle for Enhancing Health Related Outcomes from Small Scale Aquaculture Development

##### Collaborating Institutions

Lead US Institution: University of Hawaii at Hilo

Other US Collaborators: University of Rhode Island

Host Country Collaborators: Conservation International-Mexico, CIAD, CREDES, University of Sinaloa (UAS, Culican and Mazatlan Campuses), CIBNOR, Sinaloa Institute of Aquaculture (ISA), Monterey Institute of Technology, Women's Fishing Cooperatives of La Reforma, Women's Oyster Culture Cooperative of Puerto Peñasco, private sector farm managers. Selected CRSP researchers and extension agents.

##### Rationale

Improving the health and well-being of stakeholders is the fundamental justification for development of small-scale aquaculture. Striving for positive health-related impacts from large-scale endeavors is also clearly desirable. Aquaculture can affect human health through a wide variety of direct and indirect causal pathways, including but not limited to: relationship with environmental quality; use of natural resources, e.g. water, land, inputs; increasing consumption of safe, high protein food products; increases in household revenues to improve food security; and involvement of women, youth and marginalized groups.

The way in which users and resources are affected by and affect aquaculture are complex, not completely understood, and are dynamic in nature<sup>1</sup>. Workers in this area must constantly update their knowledge and understanding of the processes involved, new technology and the changing socioeconomic framework.

For these reasons, the partners involved in the Santa Maria Bay Management Project, PACRC/UHH, CRC/URI and Conservation International/Mexico (CI) have chosen to work closely with issues of aquaculture, environmental health and associated socioeconomic aspects as part of a larger Bay management initiative. Commercial shrimp culture is a major economic activity for this otherwise nearly pristine bay, but is not seen as the optimal form of the many aquaculture possibilities due to the use of sensitive habitats and potential environmental and social impacts. As a subcomponent of the Bay management plan, the partners are working to: 1) improve management of existing shrimp aquaculture through development of BMPs and zoning; and 2) work towards diversification and expansion of small-scale/subsistence aquaculture (freshwater, brackish and marine) as a supplemental livelihood. An important element of this work is the emphasis on community development and involvement of women, youth and disadvantaged groups.

CRSP stakeholder and expert panel meetings of the Arica, Asia and Latin America/Caribbean regions (2002) reveal two critical trends; 1) research and development of new aquaculture technology has been effective in laying the informational basis for development of subsistence aquaculture; and 2) the ability of researchers and extension agents to transfer and implement the outcomes of research and development has not kept pace with the rate of technological innovation nor the rapidly changing socioeconomic milieu of most developing nations and their communities. It is not uncommon for technology transfer to lag technology development in any economic sector, but the opportunity exists to significantly strengthen the collective CRSP and associated stakeholders' ability for technology transfer. The Santa Maria Bay Management Project team has identified similar issues as being major impediments to furthering their objectives of improving management and development of aquaculture as a contributor to environmental and human health.

Particularly when attempting to address issues of aquaculture and human health, a multidisciplinary and multi-institutional approach is required for effective extension strategies and programs. Aquaculture extension has traditionally been given lower priority in most regions that agricultural or public health programs with the result that its methods and reach are more limited. Aquaculture research generally receives more funding and has more personnel than does extension. To enhance the ability of researchers to understand the ramifications of their work on public health and for aquaculture extension agents to be able to more effectively transfer technology to local populations, particularly sub-sectors such as women, youth and marginalized groups, it would clearly be beneficial to team with and learn from the leading workers in public health, education, gender and indigenous sectors. Similarly, extensionists or promoters in these areas can benefit from increased understanding of the multiple benefits offered by aquaculture and can contribute to its development. Teaming of extension workers from various sectors has the potential to greatly increase the scope and impact of aquaculture extension. A second consideration is the need for extension workers in all sectors to constantly improve and expand the range of their skills, particularly for newly emerging topics or multidisciplinary topics.

### Objectives

This effort aims to:

- 1) Make positive contributions to environmental and human health through improved management of aquaculture as a component of ecosystem management and through diversification and expansion of small-scale aquaculture.
- 2) Increase the ability of aquaculture researchers and extension agents to understand and adopt extension and outreach strategies used by other sectors such as public health to increase effectiveness of small-scale aquaculture development.
- 3) Improve the understanding of aquaculture workers regarding the human health aspects of aquaculture and increase their capacity to work with health-related aquaculture topics.
- 4) Work towards building teams of extension workers from multiple sectors to develop strategies to address specific issues associated with aquaculture and health.
- 5) Develop focused, practical initiatives that couple CRSP research outcomes and extension activities which increase human health and well-being. This includes extending CRSP results to non-CRSP workers to expand expected benefits from this work.

### Strategies

This work proposes a three-pronged approach to strengthen aquaculture extension networks:

**Structured international extension exchanges** for specific topics related to aquaculture and human health. These events will be used to foster working relationships with researchers and extension agents within the aquaculture sector and from other sectors such as public health, agriculture, gender issues, indigenous community work, etc. to mutually learn about effective strategies, methods and available technologies. Invited specialists will be hosted by an in-county group comprised of representatives from multiple institutions. Participants will include representatives of the CRSP countries.

**Cross-training** during the initial part of each extension exchange will be used to set the stage for development and implementation of joint strategies. Participants will develop new and practical skills that supplement production-oriented skills traditionally the forte of aquaculture extension agents and aquaculture researchers to encompass skills needed to understand and effectively address health issues related to aquaculture.

**Develop strategies based on case studies** specifically addressing one or more topics in human health and aquaculture. These case studies will consist of actual situations present in Pacific Mexico currently under study or which are subjects of project initiatives by the group of project partners. Cases will be designed and presented to the extension exchange groups for analysis and development of strategies for immediate actions. Additionally, a few "early exercises" related to the case studies will be selected and implemented by the Mexico project partners. An "early exercise" is defined as an easily achievable activity that promises to both yield immediate results while serving as the first step or model for longer

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term initiatives. Aside from the rich learning opportunities presented by the case studies which represent real life, critical situations, potential exists to make positive and immediate impacts within the life-of-the-project and for future work should funding be available.

### Topic Areas for Case Studies

Although a significant part of the training and exchanges will focus on the philosophy, practice and basic skills of extension drawing on many sectors, specific topic areas are needed to enable participants to apply theoretical knowledge and to make measurable impacts related to health and aquaculture development. Given the extensive nature of the relationships between human health and aquaculture, it is not possible to cover the entire range, but is preferable to focus intensively and well on a few priority topics. Among the most critical topics for which rapid learning and action can take place, are the following.

#### Case Study 1: Environmental quality and human/animal health

Water quality and other aspects of environmental health are clearly impacted by aquaculture and aquaculture can also be affected by non-aquaculture sources. The Santa Maria Bay in Sinaloa, Mexico, offers a good field study and training site. Located in the heart of a major agricultural area and adjacent to communities that are growing rapidly, water quality impacts are suspected, but are not well understood. Shrimp farming is major economic activity in the area, and concerns exist that it is both impacted by declining water quality and possibly contribute to the trend. An additional issue is the desire of the stakeholders to begin culturing a wide range of bivalves (as well as local species of fish). Water quality issues are significant in considering bivalve culture because of real potential dangers to human health, the recent appearance of oyster diseases, and the increased interest in classification of waters according to ISSC<sup>2</sup> standards to allow for export to the U.S. The challenge to the participants will be to identify and define the complex range of issues, determine cost-effective methods to monitor, evaluate and mitigate impacts, and develop measures to prevent human and animal health impacts as aquaculture continues to develop. Additionally, the participants will define the steps needed to reach the point at which a system could be established to classify bivalve growing waters to allow for export to the U.S. and to prevent health impacts in the case of local consumption.

#### Case Study 2: Increasing the Involvement of Women, Youth and Indigenous Groups with Aquaculture.

The aquaculture industry of Sinaloa and Sonora is characterized by shrimp culture operations ranging from small- to large-scale and owned through a variety of means including cooperatives, *ejidos* and private ownership. The scales and modes of ownership are mixed. Nearly all of these are operated and controlled by male participants. As the State and Federal governments increasingly target aquaculture development and diversification, the issue of reaching out to groups which traditionally do not have a strong role in aquaculture is prominent. This is particularly important to effect outcomes related to household food security. Finding feasible alternatives to shrimp culture is also of importance since development of low-technology species and local species has been largely overshadowed by the shrimp "boom" of the last 15 years. Conservation International and other NGO's are working with women's cooperatives in communities surrounding Santa Maria Bay and elsewhere to develop alternative livelihoods including aquaculture. Indigenous groups are also seeking the means to practice aquaculture. Both freshwater aquaculture and some marine species (bivalves, finfish) are of interest to these populations, but extension services are generally not available to them. Technological and marketing barriers may also exist. The challenge in this case study is to develop simple and feasible strategies whereby researchers and extension agents can successfully work with these groups to begin implementing small scale aquaculture with an emphasis on non-shrimp species. This will be linked to the CRSP work in Tabasco with indigenous species and with CRSP work beginning in Sinaloa to explore polyculture of shrimp and tilapia. Additional linkages include the work with local species in the Amazon, women's fisheries and oyster culture cooperatives in Pacific Mexico, women's shrimp culture cooperatives in Nicaragua and Honduras, aquaculture conducted by the "social sector" in Mexico, and work with local communities in Africa. Work by CIAD, CIBNOR, Monterey Institute of Technology-Guaymas and UAS with development of local species will be incorporated.

Case Study 3: Food Safety and Handling; Increasing Local Consumption of Aquaculture Products. Sinaloa and particularly the Santa Maria Bay area host wild capture fisheries (shrimp and fish), a shrimp culture industry and a thriving agro-industrial sector that exports most products to the U.S. and the rest of Mexico. Initial study has shown that the wild capture fishery and the shrimp industry suffer from bottlenecks and hazards at various points in production, harvest and post-harvest processing that affect the product quality, potentially affect human health and decreases market value of the product. The agro-industrial sector is far more advanced in adopting GMPs and HACCP; these experiences may be transferred to the aquatic products sector. Issues still exist in agriculture including residual pesticides and the newly emerging GMO's. It is clear that if aquaculture expands beyond the existing shrimp culture industry, that the current infrastructure, handling practices and capacity to employ GMPs and HACCP are woefully inadequate. A secondary issue that compounds the first is that it has been difficult to mobilize and market safe and healthy aquatic products outside of the immediate production area due to inadequately developed markets, lack of value-added products and lack of understanding of safe handling procedures for small-scale local merchants. Traditional dietary practices and economic issues also come to bear here. A tertiary issue of maximum utilization and utilization of by-products also exists; the current shrimp culture industry produces a significant amount of waste in the form of shrimp heads. Increased aquaculture production may also lead to increased production of potentially useful waste. Shrimp head waste has been causing major environmental and health problems as it is generally dumped on beaches and in wetlands after processing of wild capture and cultured shrimp. Local women's cooperatives are now producing shrimp meal with the shrimp heads, but face food safety, handling and marketing challenges. Utilization of waste must be taken into account in planning for future aquaculture development. While it is predicted that the next 10 years will see Sinaloa and Sonora diversify their aquaculture sector and expand production beyond that of cultured shrimp, further development of aquaculture must consider means to improve food safety, handling and how to improve penetration of local markets to increase local consumption of aquaculture products. These weaknesses have the potential to not only affect production of standard aquaculture species such as tilapia and possibly native cichlids, but also the new, extremely high-value products that are coming into production such as callo de hacha and fugu<sup>3</sup>. The challenge to participants in this group is to identify critical issues and develop feasible short-term strategies to improve the current means of handling, processing and marketing to improve food safety and increase consumption of local products in anticipation of an aquaculture boom in the next few years.

### Design, Development and Delivery of Extension Training and Case Studies

The case studies will be developed by the project partners in consultation with local stakeholder institutions and current CRSP researchers/extension agents. Local stakeholder institutions will include institutions and individuals involved in aquaculture research and development, community development, public health, gender and indigenous issues, and agriculture extension. Input for the selection of invited extension specialists and CRSP participants will also be solicited to ensure that the collective group of non-CRSP and CRSP participant receives maximum benefit and that final project deliverables are suited for integration into other CRSP activities.

Delivery of training and case study tours will be executed by the project partners in conjunction with the current local partners.

### Geographic Scope

Most of the work will take place on the Pacific Coast of Mexico (States of Sinaloa and Sonora) but will have international representation. Mexico is fortunate in having good examples of aquaculture initiatives linked to human health. It also hosts several successful CRSP research and extension initiatives. Additionally, it is logistically well placed, being accessible and relatively inexpensive for participants from traveling from the U.S. and Latin America, and to travelers from Asia and Africa (via Mexico City or Los Angeles). Mexico is also a safe and secure area with no U.S. government travel bans or warnings. The targeted study area surrounding the Santa Maria Bay watershed area is relatively small; travel to study sites will be rapid and easy and sufficient lodging and work facilities are available. The three principal project partners are already working closely with a large number of local partners and institutions who are accustomed to, and interested in being involved in training events



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and on-the-ground activities of this nature.

While Mexico provides a good venue for training for a wide variety of reasons, it also faces challenges that exemplify similar challenges to aquaculture development faced by other regions. In particular, it is characterized by an aquaculture sector that is surprisingly under-developed given the technological and resource base of the country. While this is a product of many factors, it is in part due to a wide gap between researchers and local communities that traditional extension approaches are failing to completely bridge. It thus provides an excellent testing ground for new, multi-sectoral extension strategies that can serve as learning opportunities for other regions. Additionally, the tripartite Shrimp Culture Best Management Practices Project which is an integral part of a regional coastal management effort provides a well established framework in which to work. The primary executors of this project, CI, CRC/URI and PACRC/UHH will oversee the proposed work, but will draw on current and new partners to wide the range of topics and expertise available to the collective extension exchange.

<sup>1</sup> CRSP expert panel results (2002) from all three regions cite lack of information and understanding of the relationship between human health and aquaculture as a key issue.

<sup>2</sup> Inter-State Shellfish Sanitation Committee, which defines water quality and handling standards for shellfish culture, harvest and processing.

<sup>3</sup> Callo de hacha is a pinnid bivalve (*Atrina maura*, a pen shell) currently being put into culture by a group comprised of the three project partners, women's fishing cooperatives and the private sector. Adductor meat sells for \$30/kilo and is in demand for internal markets in Mexico City and Guadalajara and Asian markets. CIAD is working to develop the culture of fugu and other high value marine fish. Fugu is the Japanese name for pufferfish, valued for its delicate flavor but potentially dangerous to consume due to the presence of a marine toxin. The Mexican variety has very low levels of toxin making it an attractive alternative to the potentially fatal Japanese variety.



## PROJECTS UNDER DEVELOPMENT

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