

# AQUACULTURE COLLABORATIVE RESEARCH SUPPORT PROGRAM

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## TWENTY-FOURTH ANNUAL TECHNICAL REPORT

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# **AQUACULTURE COLLABORATIVE RESEARCH SUPPORT PROGRAM**

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# CONTENTS

## Environmental Impacts Analysis

Best Practices for Management of Aquaculture Pond Soils in Thailand (12EIA1).....	6
Impact of Nile Tilapia ( <i>Oreochromis niloticus</i> ) Introduction on the Indigenous Fish Species of Bangladesh and Nepal (12EIA3) .....	11
Building the Capacity of Moi University to Conduct Watershed Assessments (12EIA4).....	12
Land-use practices, policy, and tenure regimes in the Nzoia River Basin (12EIA5).....	13
Workshops on Guidelines for Developing Aquaculture Best Management Practices (12EIA6).....	18

## Sustainable Development and Food Security

Amazon Aquaculture Outreach (12SDF1).....	21
Understanding the Agricultural Knowledge System for the Development of Aquaculture in Nicaragua: Economics, Markets, and Institutions (12SDF2) .....	26
Assessing the Potential for Aquacultural Development to Promote Food Security among Indigenous People in Guatemala(12SDF3) .....	31
First Annual Sustainable Aquaculture Technology Transfer Workshop (12SDF4).....	39
5 <sup>th</sup> International Aquaculture Extension Course in the Amazon Region and 1 <sup>st</sup> Meeting for the Amazon Region Aquaculturists (12SDF5).....	40

## Production System Design and Integration

New Paradigm in Farming of Freshwater Prawn ( <i>Macrobrachium rosenbergii</i> ) with Closed and Recycle Systems: Thailand (12PSD1a).....	43
New Paradigm in Farming of Freshwater Prawn ( <i>Macrobrachium rosenbergii</i> ) with Closed and Recycle Systems: Vietnam (12PSD1b).....	44
New Paradigm in Farming of Freshwater Prawn ( <i>Macrobrachium rosenbergii</i> ) with Closed and Recycle Systems: Bangladesh (12PSD1c) .....	45
Optimization of Fertilization Regimes in Fertilized Nile Tilapia ( <i>Oreochromis niloticus</i> ) Ponds with Supplemental Feed (12PSD2) .....	46
Use of Rice Straw as a Resource for Freshwater Pond Culture (12PSD3) .....	47
Development of a Recirculating Aquaculture System Module for Family/Multi-Family Use (12PSD4) .....	49
Insulin-Like Growth Factor-1 Gene Expression as a Growth Indicator in Nile Tilapia (12PSD5) .....	50
Development of Nile Tilapia Fillets as an Export Product for the Philippines (12PSD6) .....	51
Tilapia-Shrimp Polyculture in Negros Occidental, Philippines (12PSD7).....	52
Testing Three Styles of Tilapia-Shrimp Polyculture in Tabasco, Mexico (12PSD8).....	53

## Indigenous Species Development

Broodstock Development of Amazonian Fishes (12ISD2) .....	54
Incorporation of the Native Cichlid ( <i>Petenia splendida</i> ) into Sustainable Aquaculture: Reproduction Systems, Nutrient Requirements, and Feeding Strategies (12ISD3).....	59
Broodstock Development and Larval Feeding of Amazonian Fishes(12ISD4).....	60

## Water Quality and Availability

Pond Design and Watershed Analyses Training (12WQA1) .....	66
Elimination of Methyltestosterone from Intensive Masculinization Systems: Use of Ultraviolet Irradiation of Water(12WQA2) .....	67
Elimination of Methyltestosterone from Intensive Masculinization systems: Use of Solar Irradiation and Bacterial Degradation(12WQA3).....	68
Ecological Assessment of Selected Sub-Watersheds of the Nzoia River Basin (12WQA4) .....	69
Determination of Hydrologic Baselines for the Nzoia River Basin (12WQA5) .....	70

## Economic/Risk Assessment and Social Analysis

Assessment of Coastal and Marine Aquaculture Development for Low Trophic Level Species (12ERA1) .....	71
Hydraulic, Water Quality and Social Assessment of the Nzoia Basin, Kenya (12ERA2) .....	72
Farmers Training in Tanzania (12ERA3).....	80
Ex Ante Assessment of Coastal and Marine Aquaculture Development: Charting the Strengths and Weaknesses of Low Trophic Species for Offshore Aquaculture in Developed and Developing Countries (12ERA5) .....	81
Bivalve Market Study in Pacific Mexico (12ERA6) .....	82

## Applied Technology and Extension Methodologies

Integrated Cage-Cum-Pond Culture Systems with High-Valued Climbing Perch ( <i>Anabas testudineus</i> ) in Cages Suspended in Carp Polyculture Ponds: Bangladesh (12ATE1a).....	83
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Integrated Cage-Cum-Pond Culture Systems with High-Valued African Catfish ( <i>Clarias gariepinus</i> ) in Cages Suspended in Carp Polyculture Ponds: Nepal (12ATE1b) .....	84
Integrated Cage-Cum-Pond Culture Systems with High-Valued Climbing Perch ( <i>Anabas Testudineus</i> ) in Cages Suspended in Nile Tilapia ( <i>Oreochromis niloticus</i> ) Ponds: Vietnam (12ATE1c) .....	85
Aquaculture Training for Kenyan Extension Workers, Fish Farmers, and University Students (12ATE3) .....	86
Training Local Farmers on Safe Handling of Steroids and Masculinization Techniques in Central America (12ATE4) .....	87
Establishment of the Center for Aquaculture Technology Transfer (12ATE5) .....	92
Special Sessions, Travel, and Poster Awards at 2005 and 2006 World Aquaculture Conferences and Aquaculture America 2006 (12ATE9) .....	93
Aquaculture CRSP Sponsorship of the Seventh International Symposium on Tilapia in Aquaculture (12ATE10) .....	96
Kenya Training-of-Trainers and Regionalization of Aquaculture Training Activities (12ATE11) .....	99
Post-Tsunami Training in New Aquaculture Technologies in Thailand and Indonesia (12ATE14) .....	100
ACRSP Support for International Institute for Fisheries Economics and Trade Meeting, Portsmouth 2006 (12ATE15) .....	103
<b>Seedstock Development and Availability</b>	
Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras (12SDA1) .....	104
Studies on Strategies for Increasing the Growth and Survival of African Catfish ( <i>Clarias gariepinus</i> ) Juveniles Reared for Stocking or for Use as Bait (12SDA2) .....	105
Continuation of a Selective Breeding Program for Nile Tilapia to Provide Quality Broodstock for Central America (12SDA3) .....	106
Development of Aquaculture Techniques for the Indigenous Species of Southern Mexico, <i>Centropomus undecimalis</i> : Sex Determination and Differentiation and Effects of Temperature (12SDA4) .....	107
Kenya Capacity Building: Student Research and Thesis Support(12SDA5) .....	109
<b>Fish Nutrition and Feed Technology</b>	
Effects of Native Peruvian Feedstuffs on Growth and Health of <i>Colossoma</i> and <i>Piaractus</i> (12FNF1) .....	110
Nutrition and Nutrient Utilization in Native Peruvian fishes (12FNF2) .....	111
Use of Phytochemicals as a New Method to Sex-Reverse Nile Tilapia and Tropical Garfish. Part I: Use of Aqueous Plant Extracts in Tilapia (12FNF3a) .....	117
Use of Phytochemicals as a New Method to Sex-Reverse Nile Tilapia and Tropical Garfish. Part II: Initiation of Feeding and Gonad Differentiation in Longnose Garfish (12FNF3b) .....	122
<b>Aquaculture and Human Health Impacts</b>	
Water Quality Monitoring and Identification of Pollution Sources Leading towards Classification of Bivalve Growing Waters (12AHH1) .....	126
Outreach and Planning for Implementation of Bivalve Growing Areas Classification and Related Sanitation Action Items (12AHH2) .....	127



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## BEST PRACTICES FOR MANAGEMENT OF AQUACULTURE POND SOILS IN THAILAND

*Twelfth Work Plan, Environmental Impacts Analysis 1 (12 EIA 1)  
Final Report*

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### ABSTRACT

Best management practices (BMPs) were developed for management of bottom soil in freshwater aquaculture ponds in Thailand. These practices were based on a survey of production practices in tilapia, catfish, carp, and freshwater prawn culture and upon findings of studies of physical and chemical characteristics of bottom soil from ponds of different ages. Fifteen BMPs were designed to encourage the following: pond dry-out between crops; aeration of bottoms by tillage; liming of acidic bottoms; use of sedimentation basins; responsible disposal of sediment; erosion control; application of proper construction techniques; reduction in suspended solids in effluents. The BMPs have been translated into Thai for distribution to farmers, and several of the BMPs have a high rate of adoption by Thai farmers.

### INTRODUCTION

Sustainability and protection of the environment are major issues in aquaculture (Black, 2001). Unsustainable projects are economically damaging, and they often cause environmental harm (Clay, 2004). Two main reasons for unsustainability of aquaculture operations are selection of inferior sites and application of inappropriate management procedures. Soil characteristics are a primary concern in selection of sites for pond aquaculture (Hajek and Boyd, 1994), and application of good bottom soil management is necessary for long-term operation of ponds. Moreover, attention to soil quality improves pond water quality, which in turn, favors both better production and enhanced effluent quality (Boyd, 1995).

Research funded by the Aquaculture CRSP (ACRSP) and conducted in Thailand by Auburn University researchers and collaborators from the Thailand Department of Fisheries has provided data on the physical and chemical properties of bottom soils in ponds for culture of tilapia (*Oreochromis* spp.), freshwater prawn (*Macrobrachium rosenbergii*), catfish (*Clarius* spp.), and carp (*Puntius* spp.) in freshwater areas (Thunjai et al., 2004; Wudtisin and Boyd, 2006).

Information on soil characteristics and pond management procedures from the aforementioned studies were used to develop best management practices (BMPs) for bottom soil management of freshwater aquaculture ponds in Thailand.

These BMPs will be valuable in improving the prospects for sustainable and environmentally responsible pond aquaculture in Thailand and other nations.

### MATERIALS AND METHODS

Data on site characteristics, pond design, culture methods, and physical and chemical characteristics of pond bottom soils related to culture of tilapia, catfish, carp, and freshwater prawn (Thunjai et al., 2004; Wudtisin and Boyd, 2006) were examined to determine similarities and differences among culture systems and methods for the four species. The data also were evaluated to ascertain if the suitability of pond bottom soils for aquaculture was deteriorating as ponds aged. Information from the studies of pond bottom soils in freshwater ponds in Thailand (Thunjai et al., 2004; Wudtisin and Boyd, 2006) was used to develop BMPs for pond bottom soil management.

The pond bottom soil BMPs were translated to Thai by Dr. Idsariya Wudtisin, a former, ACRSP-supported student at Auburn University and now a faculty member at Kasetsart University in Bangkok.

The US PI traveled to Thailand three times between January and June 2006. He visited 12 fish farms and made an evaluation of the adoption of pond bottom soil BMPs.

RESULTS

Based on findings of the studies by Thunjai et al. (2004) and Wudtisn and Boyd (2006), the main differences among the culture systems for the four species were as follows:

- Natural soil acidity was greater in the area for tilapia culture than in the areas for culture of the other three species.
- Ponds were constructed in areas where soil quality was suitable for pond construction and aquaculture. Areas of extremely sandy soil and organic soil were avoided as pond sites. Natural acidity of soils was mitigated by using liming materials.
- Carp and prawn ponds usually were larger in area than tilapia and catfish ponds.
- Most ponds were drained annually, but carp ponds integrated with chicken production were only drained at 3- to 5-year intervals.
- Nutrient and organic matter inputs increased in the order freshwater prawn < carp < tilapia < catfish.
- There were some differences between bottom soil characteristics and culture species, and soil characteristics changed with pond age. However, these differences and changes were not great enough to require different soil management according to culture species.

Because pond management and soil physical and chemical features of bottom soil were relatively similar, the BMPs were designed for general pond soil management without regards to species. Fifteen individual BMPs were prepared (Table 1). The Thai language version of the BMPs is provided as Appendix 1 to this report. The estimated adoption rate of the BMPs as of June 2006 is provided in Table 2.

DISCUSSION

**Best Management Practices for Maintaining Pond Soil Quality**

*BMP1. After ponds are drained for harvest, bottoms should be dried for 2 to 3 weeks before refilling with water.*

Accumulation of fresh, labile organic matter in pond bottoms can lead to high rates of microbial respiration. Sediment usually is anaerobic below a depth of a few centimeters in ponds for extensive production. In intensive ponds, only the upper few millimeters of sediment are aerobic (Munsiri et al., 1995), and if organic matter inputs are especially great, microbial respiration can result in dissolved oxygen depletion at the sediment-water interface. This phenomenon is undesirable because potentially toxic compounds from anaerobic metabolism of bacteria, e.g., nitrite, ferrous iron, hydrogen sulfide, and organic fermentation products, can enter the water.

Table 1. List of best management practices (BMPs) for pond bottom soils.

BMP No.	Statement of BMP
1	After ponds are drained for harvest, bottoms should be dried for 2 to 3 weeks before refilling with water.
2	The bottoms of empty ponds should be tilled to a depth of 10 to 15 cm with a disk harrow to improve soil aeration.
3	Pond bottom soils should be treated with liming materials to increase soil pH to between 7.5 and 8.
4	Sediment should be removed from ponds before it becomes deep enough to interfere with pond management procedures.
5	Use sedimentation basins to remove suspended soil particles from incoming water.
6	Install vegetation, stone, or other cover on pond embankments to reduce the potential for erosion.
7	Use proper side slopes and compaction when constructing new ponds or renovating old ones.
8	In ponds with mechanical aeration, install aerators to prevent water currents from eroding insides of embankments. Install rip-rap (stone) on bottom in front of aerators to prevent scouring of the pond bottom. If bottoms of heavily aerated ponds are tilled between crops, compact bottoms with heavy roller before refilling.
9	Do not leave ponds empty longer than necessary during rainy weather to prevent erosion of soil from shallow area with deposition of soil in deeper areas.
10	Do not allow livestock to walk on pond embankments or wade in shallow water edges.
11	Avoid operating equipment that will cause ruts and other inundations in pond bottoms.
12	Design and construct discharge canals to minimize bottom scouring and erosion of side slopes. This should include installation of grass cover and stone reinforcement of erosion prone areas in canals.
13	Do not use water jets to wash pond bottoms by hydraulic pressure as is sometimes done by marine shrimp farmers in Thailand.
14	Pass pond effluents through a settling basin to remove coarse suspended solids before final discharge into natural waters.
15	Dispose of sediment removed from pond bottoms or settling basins in a responsible manner.

Table 2. Use of bottom soil best management practices (BMPs) by operators of freshwater aquaculture ponds in Thailand. Twelve farms were included in the survey.

BMP No.	Estimated rate of adoption (%)
1	83.3
2	58.3
3	75.0
4	100.0
5	25.0
6	25.0
7	16.6
8	Not applicable <sup>1</sup>
9	Could not be determined <sup>2</sup>
10	83.3
11	Could not be determined <sup>2</sup>
12	25.0
13	100.0
14	0.0
15	50.0

<sup>1</sup>None of the ponds in the sample were aerated.  
<sup>2</sup>Ponds were full when visited.

The concentration of labile organic matter increases during an aquaculture crop usually reaching a maximum near harvest time. When ponds are drained, some of the labile organic matter is suspended by outflowing water and removed from ponds (Ayub et al., 1993), but much remains. Drying of the pond bottom allows air to enter into pore spaces and cracks in the soil to accelerate aerobic microbial activity and oxidize reduced compounds (Wurtz, 1960; Boyd, 1995). This is beneficial in reducing the amount of labile organic matter present at the beginning of the next crop. It also oxidizes inorganic compounds so they can be used again as sources of oxygen by bacteria within the anaerobic zone of sediment. The labile organic fraction and reduced inorganic compounds can be oxidized within two or three weeks. The refractory organic matter usually does not decompose fast enough to cause anaerobic conditions at the sediment-water interface (Boyd, 1995).

Freshwater fish and prawn farmers in Thailand practiced bottom dry-out between crops when ponds were drained for harvest. The reported dry-out period of two to three weeks is probably adequate in ponds without deep sediment. Drying for longer periods usually is not beneficial because soils become so dry that bacterial activity is retarded for lack of moisture (Boyd and Teichert-Coddington, 1994). Sediment deeper than 15 or 20 cm will take several months to dry.

*BMP 2. The bottoms of empty ponds should be tilled to a depth of 10 to 15 cm with a disk harrow to improve soil aeration.*  
 Tillage greatly increases the exposure of pond soil to the air to accelerate drying and oxidation. Tillage is especially important in soils with clay content over 20 or 30%. Although such soils often crack into columnar blocks upon drying (Pettry and Switzer, 1993), air cannot enter into the blocks (Boyd, 1995). Tillage

pulverizes these blocks of soil to allow the wet soil from inside the blocks to dry and oxidize. Farmers interviewed in this study did not till pond bottoms between crops. Many of the ponds had clay content above 20%, and tilling of pond bottoms would likely be beneficial in freshwater aquaculture in Thailand.

*BMP 3. Pond bottom soils should be treated with liming materials to increase soil pH to between 7.5 and 8.*  
 Acidic bottom soils are associated with low total alkalinity in pond waters (Boyd and Tucker, 1998). Aquaculture ponds with low alkalinity waters do not have a large reserve of carbon dioxide, but they have high concentrations of nitrogen and phosphorus. Phytoplankton blooms that develop in ponds with low alkalinity water cause an excessively high pH by depleting the free carbon dioxide supply. Moreover, acidic conditions in bottom soils limit the growth of benthic organisms important as natural food for culture species. Bacteria also are limited by low pH, and organic matter may accumulate in pond bottoms and nutrient recycling will be slow. Pillai and Boyd (1985) presented a lime requirement method for determining the liming rate for aquaculture ponds. However, if this method cannot be used, the lime requirement could be based on soil pH. Ponds with soil pH of 7.5 or above would not need lime, and 500 kg ha<sup>-1</sup> of liming material should be applied for each 0.5 unit decline in pH. For example, at pH 7, apply 500 kg ha<sup>-1</sup> and at pH 6.5, apply 1,000 kg ha<sup>-1</sup>. Where producers do not have a means of measuring soil pH, an initial liming rate of 2,000 kg ha<sup>-1</sup> could be used, and afterwards, 500 kg ha<sup>-1</sup> could be applied annually.

Ponds for catfish, freshwater prawns, and carp would benefit from applications of liming materials for soil pH was below 7 in many of them. However, tilapia ponds in Thailand are heavily limed, and in some cases, applications could be reduced (Thunjai et al., 2004).

*BMP 4. Sediment should be removed from ponds before it becomes deep enough to interfere with pond management procedures.*  
 Part of the sediment in ponds has its origin in suspended solids in incoming water that settle to the pond bottom. Erosion of pond embankments and shallow areas by waves and water currents suspends soil particles that tend to settle in deep areas. Organic matter from plankton, uneaten fish feed, and feces also become sediment (Boyd, 1995). Sediment is comprised mainly of mineral soil particles, but organic matter deposits onto the sediment as a flocculent layer and is gradually mixed into the sediment mass (Munsiri et al., 1995).

Deep sediment has several undesirable effects in ponds (Boyd, 1995; Steeby et al., 2001). Feed pellets may sink into it and not be eaten by the culture animals. Feed pellets decompose rapidly and may cause localized zones of especially low redox potential. Soft sediment fills nets and seines during harvest making it difficult to pull them. A large volume of mud in nets or seines can injure aquatic animals and also make them difficult to remove. When ponds are dried between crops, areas with sediment deeper than 15 to 20 cm usually will not dry out.

Fish and freshwater prawn farmers in Thailand practice sediment removal. Nevertheless, some ponds, and especially carp ponds, had deep sediment. More attention to sediment removal would be beneficial in some ponds.

Some farmers interviewed in this study dragged chains over pond bottoms or used suction devices to remove soft sediment



during crops. Previous studies showed little benefit of such practices (Beveridge et al., 1994; Gomes, 2003), and disturbance of an anaerobic sediment might release harmful amounts of metabolites into the water. Microbial products also are applied to ponds to improve the quality of soft sediment. A recent review (Boyd and Silapajarn, 2005) did not find documentation of soil and water quality benefits following applications of microbial products. Therefore, sediment removal appears to be the most effective way of dealing with soft sediment in aquaculture ponds.

*BMP 5. Use sedimentation basins to remove suspended soil particles from incoming water.*

In some cases, the water supply for ponds was highly turbid with suspended soil particles from erosion on watersheds. Because these particles will settle in ponds, it is beneficial to install a sedimentation basin to remove coarse suspended particles before they enter ponds. A settling time of one or two hours can be beneficial, but for best results, a settling time of four hours or more should be provided (Boyd, 1995).

*BMP 6. Install vegetation, stone, or other cover on pond embankments to reduce the potential for erosion.*

Erosion of pond embankments can be a major source of settleable solids in ponds. Soil particles may be suspended by waves and currents in ponds and by rain falling on bare soil of embankments. Installation of cover to avoid erosion reduces the sediment load to ponds and protects the embankments.

*BMP 7. Use proper side slopes and compaction when constructing new ponds or renovating old ones.*

This practice will reduce the tendency of earthwork to erode and reduce the internal sediment load in ponds. It also reduces maintenance costs for repairing embankments.

Recommended side slopes for embankments made of clay, clayey sand, clayey gravel, sandy clay, silty sand, or silty gravel are 3:1 (horizontal:vertical) on the wet side and 2:1 on the dry side. Slopes of 3:1 should be provided on both sides of an embankment made of silty clay or clayey silt. Where well-graded soil has been compacted properly, the side slopes may be 1:1 or 2:1 on both sides (Yoo and Boyd, 1993). Even with proper side slopes, vegetative cover or rock must be provided to avoid erosion.

*BMP 8. In ponds with mechanical aeration, install aerators to prevent water currents from eroding insides of embankments. Install rip-rap (stone) on bottom in front of aerators to prevent scouring of the pond bottom. If bottoms of heavily aerated ponds are tilled between crops, compact bottoms with heavy roller before refilling.*

Mechanical aerators induced strong water currents in ponds. If the aerators are placed too close to embankments, currents flowing parallel to embankments may cause erosion. Water currents directed at embankments can cause even greater erosion. There also is a tendency for increased erosion of the pond bottom in front of aerators. Tilling of pond bottoms loosens the soil making it more susceptible to erosion by water currents generated by aerators.

Farmers interviewed in this study did not use mechanical aerators. However, mechanical aeration is a very effective practice that improves water quality and allows greater production. In the future, freshwater aquaculturists in Thailand will probably use aeration. It is commonly used by marine shrimp farmers in Thailand.

*BMP 9. Do not leave ponds empty longer than necessary during rainy weather to prevent erosion of soil from shallow area with deposition of soil in deeper areas.*

Rainfall erosion can cause serious alteration of empty pond bottoms. If drains in empty ponds are left open, water with high concentrations of suspended soil particles will flow from ponds into natural water courses.

*BMP 10. Do not allow livestock to walk on pond embankments or wade in shallow water edges.*

Livestock can make paths by walking along the same route each day. These paths often are sites of erosion that can develop into small gullies.

*BMP 11. Avoid operating equipment that will cause ruts and other inundations in pond bottoms.*

Ruts or other depressions in pond bottoms often fill with soft sediment. They also create areas that cannot be drained and dried completely.

### Best Management Practices for Preventing Off-site Impacts

Aquaculture in ponds can cause negative, off-site environmental effects. For example, effluents contain suspended solids, nutrients, and organic matter and they can cause turbidity, sedimentation, and eutrophication in receiving water bodies (Goldburg and Triplett, 1997). Sediment removed from ponds may be discarded in piles on vacant land. This practice can result in destruction of vegetation and other terrestrial ecological nuisances. Moreover, erosion of sediment piles can cause turbidity and sedimentation in nearby water bodies. Best management practices also can be used to avoid off-site environmental impacts. Some soil-related practices that would be useful in Thailand are listed below:

*BMP 12. Design and construct discharge canals to minimize bottom scouring and erosion of side slopes. This should include installation of grass cover and stone reinforcement of erosion prone areas in canals.* Rather complex engineering practices are necessary to minimize erosion in earthen canals (Yoo and Boyd, 1993). However, the basic principle is to make the channel cross section large enough to prevent excessive water velocity and make the channel side slopes gentle enough to prevent erosion.

*BMP 13. Do not use water jets to wash pond bottoms by hydraulic pressure as is sometimes done by marine shrimp farmers in Thailand.* The practice of washing pond bottoms is a method of sediment removal. However, the material suspended by water pressure is discharged into canals. The effluent from pond cleaning can cause turbidity, excessive oxygen demand and sedimentation in canals. This will lessen the quality of water for other water users.

*BMP 14. Pass pond effluents through a settling basin to remove coarse suspended solids before final discharge into natural waters.* Soil particles resuspended when ponds are drained for harvest cause high total suspended solids concentrations in pond effluents. Settling basins can remove most of these solids and avoid sedimentation problems in the receiving water body.

*BMP 15. Dispose of sediment removed from pond bottoms or settling basins in a responsible manner.*

Sediment should be placed back over the areas on pond bottoms and embankments from which it originated if practical. Pond sediment has a high nutrient content, so it could be spread over and incorporated into agricultural soil. Pond sedi-

ment also can be used as earthfill. If it is spread over the land, grass cover should be established to prevent erosion.

The quality of bottom soils in ponds for the three types of aquaculture was generally good. However, it could be improved and external environmental impacts prevented through the use of BMPs. This would help improve the sustainability of tilapia, catfish, freshwater prawn, and carp farming in Thailand.

#### Adoption of BMPs

Some of the BMPs have traditionally been used by Thai fish and shrimp farms while others were possibly adopted as a result of ACRSP research in Thailand. We were unable to distinguish which practices resulted directly from the ACRSP. However, the average adoption of BMPs (omitting BMPs 8, 9, and 10 that were either not applicable or could not be measured) was 53.4%.

#### CONCLUSIONS

- (1) Management practices and bottom soil characteristics do not differ greatly among culture ponds for tilapia, carp, catfish, and freshwater prawn in Thailand.
- (2) General pond bottom soil best management practices (BMPs) were designed for freshwater aquaculture in Thailand.
- (3) These BMPs focused on maintaining good bottom soil quality by dry-out, liming, tillage, and periodic sediment removal. They also emphasized erosion control and removal of excessive suspended solids from the water supply and from farm effluent.
- (4) The BMPs should be applicable to pond aquaculture in other nations.

#### ANTICIPATED BENEFITS

The bottom soil BMPs will be useful in improving the management of freshwater aquaculture ponds in Thailand. Application of the BMPs will protect the earthwork of ponds from erosion, lessen the accumulation of sediment in ponds, and assure reasonable bottom soil quality. The BMPs also can present off-site problems with turbidity and sediment accumulation in bodies of water receiving pond effluents. The BMPs should improve the prospects for sustainability of pond culture in Thailand. The BMPs are general, and they should be useful in other nations. Some of the BMPs may be included in effluent regulations for pond aquaculture to lessen contamination of receiving water bodies with suspended soil particles and resulting turbidity. In the future, the BMPs will be useful to producers seeking to comply with aquaculture certification standards.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## IMPACT OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) INTRODUCTION ON THE INDIGENOUS FISH SPECIES OF BANGLADESH AND NEPAL

*Twelfth Work Plan, Environmental Impacts Analysis 3 (12EIA3)*  
*Abstract*

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### ABSTRACT

The objective of this study is to assess the impact of the introduction of mixed-sex and male mono-sex Nile tilapia on three important indigenous fish species of Bangladesh and Nepal. The study is being conducted in small ponds where changes in population structure and recruitment are being assessed over time. This study consists of three experiments, two of which are being conducted in Bangladesh and the other in Nepal.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## **BUILDING THE CAPACITY OF MOI UNIVERSITY TO CONDUCT WATERSHED ASSESSMENTS**

*Twelfth Work Plan Economic/Risk Assessment and Social Analysis 4 (12EIA4)  
Abstract*

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### **ABSTRACT**

The diverse knowledge of social scientists, hydrologists, and agronomists is crucial for a watershed assessment team. Diversity is important for both host country and US PI counterparts. Remote sensing techniques coupled with ground truth data by the social scientist, hydrologist, and crop scientist make a comprehensive assessment a doable undertaking. The proposed assessment table was found to produce needed results. We found the methodology was not inherently linear. Due to the great distances and diversity of the team members, the aspects of the table are developed with a degree of opportunism. The process is in the general sense linear. Although the analyses laboratory has been slow to form and become recognizable, the expertise base is solidifying and an excellent foundation for future work is in place.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## LAND-USE PRACTICES, POLICY, AND TENURE REGIMES IN THE NZOIA RIVER BASIN

*Twelfth Work Plan, Environmental Impacts Analysis 5 (12EIA5)  
Final Report*

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### ABSTRACT

Identification of current soil and water conditions and conservation practices is an initial step toward development of appropriate technologies for land use management plans. In western Kenya, land use activities for two villages along the Kapolet River and two locations along the Moiben River typify maize production potential and its cultural significance. Research objectives include identification of the:

- 1) Kinds of soil and water conservation (SWC) practices in use;
- 2) Factors that influence the adoption of SWC practices; and
- 3) Characteristics of those who adopt SWC practices.

Data were acquired in each location through participatory rural appraisal activities ( $n=4$ ), informant interviews ( $n=8$ ), household questionnaires ( $n=172$ ), and personal observation. Household respondents indicated that 59% apply organic fertilizer, 56% plant grass strips, 29% build terraces, and 29% leave land fallow. Significant relationships existed between the use of SWC methods and region-specific household characteristics (e.g., wealth, number and type of livestock, acreage and land ownership, and perception of soil conditions). People who adopted SWC practices had an interest and knowledge of SWC purpose and implementation. Steep slope conditions and financial resources also influenced adoption of SWC practices. If implemented, specific SWC methods act to prevent erosion and subsequent loss of soil fertility. Identifying opportunities to increase farmer's capacity to adopt conservation practices will contribute to meeting the demands for high maize production from this region, while also improving local and regional soil and water quality.

INTRODUCTION

Soil and water conservation (SWC) adoption often reflects individuals’ access to and use of resources. In Kenya, concerns with soil fertility and soil retention, and water quality and availability have been documented with changing political regimes, shifting agricultural demands, and environmental degradation. SWC studies in Sub-Saharan Africa suggest that adoption is influenced by knowledge and perceptions of soil fertility and erosion, access to extension services, land security, education level, wealth status, gender, topographic conditions, and the individuals’ cost/benefit analysis of implementation (Bandre and Batta, 1999; Bewket and Sterk, 2002; Boyd et al., 2000; Hanson, 2000; Kerr and Sanghi, 1997; Ovuka, 2000a; Ovuka, 2000b; Place et al., 2003; Shepherd et al., 2000). Individual livelihood strategies determine whether farmers implement SWC practices and this is influenced by individuals’ social, physical, and demographic position (Boyd et al., 2000). The difference in these variables and the interactions among them creates heterogeneous land use strategies and the available assets to promote SWC adoption (Place et al., 2001).

METHODS AND MATERIALS

Data analyses included a review of quantitative and qualitative data collected through personal observations, household questionnaires (n=172), and key informant interviews (n=8). A collective summary of landscape conditions is described from household questionnaires representing the Kapolet and Moiben regions. Quantitative analyses included percentages and ranks of SWC use in each region. The influence of household characteristics and physical traits on the percent adoption of manure, terraces, grass strips, and fallows was determined by chi-square test of independence and evaluated at α=0.05 level. All statistical data analyses were performed using SPSS version 12.0 (SPSS 2003). Cramer’s V, a measure of association, was used to detect relationships between multiple categorical variables to include education levels and wealth status levels. Differences in sample means for acreage and livestock numbers were established through independent samples t-test and analysis of variance for multiple variables to determine if relationships existed between the dependent variable of SWC adoption and independent variables such as demographic and site descriptive characteristics. To understand SWC adoption benefits, constraints, and education resources, qualitative information was coded allowing for frequency and ranks of conditions across the study sites.

RESULTS

In the Kapolet region, soil and water conservation practices in use according to the Ministry of Agriculture (MoA) extension officer and Vi Agroforestry (a nongovernmental organization) extension personnel, included grass strips, terracing, erosion trenches, strip cropping, alley farming or agroforestry, road water diversion, trash lines (crop residues), non-tillage, fallow lands and the use of animal manure as fertilizer.

Household questionnaire respondents indicated that 63.7% used grass strips, 51.6% used animal manure as fertilizer, 31.9% used terraces, and 22% used fallowing (Table 1). Native plant use, intercropping and composting were also important soil and water management activities indicated by household respondents. In the Moiben region, soil and water conservation practices in use as noted by the MoA extension officer, household ques-

Table 1. Soil and Water Management- Household Questionnaire Summary

	Kapolet (n=91)			Moiben (n=81)		
	%			%		
SW MANAGEMENT ACTIVITIES						
manure	51.6			66.7		
grass strips	63.7			46.9		
terraces	31.9			24.7		
fallow	22.0			37.0		
inorganic fertilizer	68.1			90.1		
SW DECISIONS AND MANAGEMENT*						
% usage change	-	same	+	-	same	+
manure	6.6	5.5	39.6	2.5	16.0	46.9
grass strips	4.4	18.7	39.6	1.2	25.9	17.3
terraces	1.1	12.1	19.8	0	9.9	14.8
fallow	7.7	11.0	3.3	7.4	18.5	11.1
inorganic fertilizer	19.8	12.1	36.3	13.6	27.2	49.4

\* increase: +  
decrease: -

tionnaires, and personal observations, included grass strips, erosion trenches, fallow lands, hedgerows with native plants, road water diversion, and reforestation attempts. Household questionnaire respondents indicated that 66.7% used animal manure as fertilizer, 46.9% used grass strips, 37% used fallowing, and 24.7% used terraces (Table 1). Native plant use, hedgerows, and drainage ditches were also important soil and water management activities indicated by household respondents in the Moiben region.

Household questionnaire respondents indicated SWC adoption was influenced by access to information and outside assistance, land ownership, land size, limited financial and physical implementation resources, livestock numbers, negligence, perceptions of sustainability, and/or effectiveness, and topography. A summary of significant relationships between the targeted land use activities and household and physical variables are listed in Table 2. M/K indicates the area with the higher percentage of the statistically significant difference.

Characteristics of SWC Adopters

Data collected through individual household interviews enabled the analysis of associations between SWC adoption and specific traits of individual farmers and/or their farming conditions. In all four study sites, personal interest and having knowledge of SWC practices were the top two ranked characteristics of SWC users as described by household respondents (Table 3). Slope conditions lent to the need for SWC implemen-

## ENVIRONMENTAL IMPACTS ANALYSIS

Table 2. Significant SWC Variables- Household Respondent Results  
M=Moiben, K= Kapolet, x= significant relationship at  $p \leq .05$

	Region	Manure	Grass strips	Terraces	Fallow	Fertilizer
Region		M	K		M	x
Acreage	M			x		x
Cows	M	x	x (Kapolet)			
Education		x				x
Erosion			x		x	
Soil fertility	K		x			x
Title	M	x		x		x
Topography						x
Wealth status		x				x

tation and having financial resources for implementation were the next ranked traits of SWC adopters. Household respondents described characteristics of other people that did not adopt SWC practices as those people who lacked understanding and implementation knowledge, had no personal interest because they believed their land did not need SWC, and demonstrated negligence (those capable with the knowledge and need, yet still not implementing SWC). Table 3 lists the characteristics of people who are either adopters or non-adopters.

### SWC Education Sources

The adoption of SWC methods also depended on where people learned about these activities. People sought information from people they trusted, from those with whom they had proven past relationships, and from those whom have demonstrated success. Sources for learning about SWC in both regions included MoA extension trainings, information acquired in primary and secondary schools, farmer training schools, information from friends, family, and neighbors, repeated methods used on colonial farms and self-taught trial and error. SWC education source frequencies, ranks, and extension services for each region are listed in Table 4.

## DISCUSSION

The conditions that influenced SWC adoption in the Kapolet and Moiben regions corresponded to assessments previously conducted in Western Kenya (Hansen, 2000; Place et al., 2003; Shepherd et al., 2000), Burkina Faso (Bandre and Batta, 1999), Ethiopia (Bewket and Sterk, 2002) Tanzania and Uganda (Boyd et al., 2000). The principal variables in this study that acted independently or in combination to influence SWC adoption included, personal interest, access to information, wealth variables (e.g., livestock, land security), slope conditions, and perceptions and /or effectiveness of the practice.

This study looked specifically at the adoption of organic and inorganic fertilizer, grass strips, terraces, and fallowing practices and their relationships to influencing variables (Table 2). Differences between percent use of manure, grass strips, and

Table 3. SWC Adopter Characteristics- Household Questionnaire Summary

	SWC Users %	SWC Non-Users %
Personal interest	36.1	13.9
Knowledge	33.8	43.6
Negligence	0.0	12.1
Land ownership	0.7	3.6
Land size	2.2	7.3
Topography	13.2	6.1
Financial	10.3	6.1
Physical constraints	2.2	1.2
Cooperation between land users	0.0	1.2

fallowings occurred between the regions, although no difference was detected for use of terraces. Manure was associated with indicators of wealth. Terraces were associated with land security. Grass strips were more likely used by people who were diversifying their farming strategy with dairy cows. The benefits and constraints of these SWC practices were site and activity specific, which influenced adoption rates as described by household respondents. Grass strips were associated with farmers who believed their soil fertility increased and erosion was controlled through their use. Fallowing was also associated with people who did not think erosion was a problem because they felt their land use practices prevented erosion.

The differences between regions and the types of SWC implemented reflected available assets and contributed to farm strat-

Table 4. SWC Education Sources- Household & Key Informant Interview Results

	Kapolet		Moiben	
	%	rank	%	rank
<b>SWC EDUCATION SOURCES*</b>				
Friends, family, neighbors	25.3	2	45.7	1
Extension	37.4	1	24.7	3
School	9.9	4	25.9	2
Self-taught	11	3	18.5	4
Copy previous SWC Method	8.8	5	3.7	6
Farmer training schools	1.1	6	9.9	5
<b>SWC EXTENSION SERVICES</b>				
	<u>MoA extension &amp; Vi Agroforestry /LIFE</u>		<u>MoA extension</u>	
	-community education		-individual farm consultations	
	-organic farming		-fertilizer /seed recommendations	
	-marketing cooperatives			
	-money management groups			
	-agroforestry			
	-fertilizer / seed recommendations			
	-SWC demonstrations			

\* multiple responses % may not equal 100

### CONCLUSIONS

egy heterogeneity. The inclusion of SWC in farm strategies was attributed to individual limitations (e.g., land, labor, financial capital) and personal interest given their assets, land conditions, and knowledge of SWC implementation and management. Outreach capabilities and the willingness of community participants to accept new information were unique to each region and therefore influenced the adoption of SWC practices. Both regions indicated SWC knowledge was a limitation to adoption; however, each region had different sources for this information.

SWC adoption rates reflected how interactions among the variables influenced adoption in each region. Characteristics of wealth had the most influence on adoption, both directly and indirectly. Some practices were not directly linked to wealth status but were influenced by characteristics of wealth (e.g., education, title ownership, livestock ownership, acreage) (Table 2). SWC activities are long-term investments that require an initial investment of time, labor, and money. These requirements make asset deficient farmers less capable of adopting SWC because of the delayed benefit returns. Manure, grass strip, and terrace use were all linked to at least one asset-attributing variable that increased SWC adoption rates (Table 2). Exceeding investment poverty levels by accumulating assets, which enables SWC adoption (Reardon and Vosti, 1995) is often site specific and this was demonstrated by the interrelationships of the variables contributing to grass strip adoption in the Kapolet region.

Influencing variables that affect SWC adoption of one farmer and not another, suggests the need for providing a variety of SWC technology options (Place et al., 2001). Higher adoption rates of grass strips and manure use in the Kapolet and Moiben regions reflect the multiple purposes of successful SWC practices. Grass strip and manure use provide dual benefits and act as assets that promoted accumulation of other assets and may improve overall livelihood conditions. Grass strips represent an erosion control method with minimal investment in seed costs that could be used in all topographic conditions, while simultaneously providing feed for livestock. Manure use contributes to long-term soil fertility improvements by incorporating organic manure into the soil, which reduces the need for chemical fertilizer whose nutrient value is likely to be lost with erosion, and heavy rains. Widespread manure use is limited by supply and application labor. However, most households have livestock from which manure compost could be made and applied to household compound gardens. Providing a range of SWC opportunities with visible short-term benefits for both small and large landholdings may increase adoption rates. The subsistence farming communities within the Kapolet and Moiben regions reflect a trend in decreasing farm plot size. The smaller farming plots need SWC practices that are beneficial to a landholder that is likely to be less educated and less affluent than larger landholders.

The development of appropriate SWC technologies that reach a greater percentage of stakeholders may show that SWC is not an activity reserved only for the wealthy landholders. Reaching a larger number of stakeholders with viable SWC adoptions may have a greater impact on overall soil and water



conditions because land management schemes in these areas were based on small subsistence farms.

Dissemination of SWC information must account for the method by which farmers receive information. In the Kapolet region, extension officers successfully disseminated information. However, in the Moiben region, information was more likely to be disseminated through friends and family members. Further evaluation of the most effective way to disseminate SWC information in each of these communities may help to develop land use management plans. Improved extension services that work within a community's cultural communication system may improve SWC adoption rates by providing knowledge about different types of SWC practices, their purposes, and the tools needed for implementation.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## WORKSHOPS ON GUIDELINES FOR DEVELOPING AQUACULTURE BEST MANAGEMENT PRACTICES

*Twelfth Work Plan, Environmental Impacts Analysis 6 (12EIA6)  
Final Report*

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### ABSTRACT

Workshops on procedures for developing best management practices for lessening negative environmental impacts of pond aquaculture were convened in Grahamstown, South Africa and Piracicaba, Brazil. There were 78 registered participants in South Africa and 38 in Brazil. A manuscript that outlines the issues related to aquaculture and the environment and the processes that should be followed in drafting, revising, presenting and implementing BMPs was prepared for use in the workshops. The workshop could easily be presented at other locations using the material and experience from the efforts in South Africa and Brazil as a model.

### INTRODUCTION

The aquaculture industry has been embroiled in controversy about its environmental record, and the long-term sustainability of some activities has been questioned (Pillay, 1996). There are documented cases of environmental degradation caused by aquaculture (Dierberg and Kiattisimkul, 1996; Naylor et al., 2000; Boyd and Tucker, 1998). Abandoned shrimp-farming projects comprising thousands of hectares may be found in coastal areas of several countries (Boyd and Clay, 1998), but there also are less publicized cases of abandoned fish farms in freshwater areas of several nations.

In response to criticism from the environmental community, aquaculture organizations have begun to develop codes of practice and best management practices (BMPs) for voluntary adoption by their members or clients. As one might expect, these instruments are highly variable in content and format. However, the better ones are developed through a collaborative, transparent stakeholder process involving several rounds of review and revision (Boyd, 2003a,b; Boyd et al., 2003). The BMPs are designed to prevent or mitigate possible negative impacts identified in an environmental assessment of the industry (Boyd et al., 2000). Moreover, suggestions are included to aid in the implementation of the BMPs (Donovan, 1997; Boyd et al., 2003, 2004).

Consumers are becoming more environmentally aware and seeking products with a good environmental record (Seafood Choices Alliance, 2003). An increasing number of seafood brokers and institutional buyers of fish, shrimp, and other aquatic products are seeking to do business with farms that use environmentally-responsible culture methods. This trend is expected to continue, and BMPs will become an important environmental and marketing tool in aquaculture.

The particular combination of BMPs needed to assure environmentally-responsible operations varies among culture techniques and species. Different combinations of BMPs are necessary in different regions and at different sites, and BMP development should be done at a relatively local level. Nevertheless, the same general process can be used in developing best management practices, and guidelines for developing aquaculture BMP need to be presented to the aquaculture industry.

Aquaculture is a well-established endeavor in Brazil and there is much interest in developing a significant aquaculture industry in South Africa. Workshops to present procedures and process that should be followed in developing aquaculture BMPs will be valuable to future efforts to improve the environ-

mental performance of aquaculture in these two nations. The experience gained presenting the two workshops will be used to develop a manual on how to develop BMPs that will be useful throughout the global aquaculture industry.

### MATERIALS AND METHODS

Two workshops were convened. One was held in conjunction with the 7<sup>th</sup> Annual Meeting of the Aquaculture Association of Southern Africa at Grahamstown, South Africa on 15 and 16 September 2005. The other was conducted at the Luiz de Queiroz Superior School of Agriculture of the State University of São Paulo at Piracicaba, Brazil from 28 to 30 March 2006.

The general outline of the workshops is provided below:

- Introduction
- Aquaculture and the Environment
- Codes of Conduct and BMPs: An Introduction
- Presentations about National Aquaculture
  - Production statistics and future trends
  - Market considerations
  - Culture systems
  - Environmental impacts
    - Aquaculturist's opinion
    - NGO concerns
  - Aquaculture regulations
- Environmental Impact Assessment in Aquaculture
- Site Evaluation Issues
- Developing Draft BMPs to Prevent or Mitigate Impacts
- Involving Stakeholders in Review and Refinement of BMPs
- Guidelines for Implementation of BMPs
- Application of BMPs
- Questions and Answers

Experts from the host countries were invited to speak about the status of national aquaculture, and especially about the culture methods used. Host country specialists were invited to speak about national opinions related to aquaculture and the environment and aquaculture national aquaculture regulations. The US PIs and the Host Country PIs were responsible for presenting the remainder of the workshop.

A wide range of stakeholders were invited to include fish and shrimp producers, feed company representatives, aquaculture research and extension specialists, NGO representatives, governmental fisheries, aquaculture, and environmental officials, and news reporters. In addition, undergraduate and graduate students in fisheries, aquaculture, and environmental sciences were invited.

To supplement the presentations of the workshop, a manuscript was prepared by the PIs. This document outlined the issues related to aquaculture and the environment, defined processes that could be followed to allow input from a wide range of stakeholders, and discussed procedures for drafting, reviewing, presenting, and implementing best management practices (BMPs). The manuscript has been revised to correct shortcomings that were found during the workshops.

### RESULTS

Seventy-eight individuals registered for the workshop in South Africa. However, because it was held concurrently with other

activities of the Aquaculture Association of South Africa, participants tended to alternate between other activities and the workshop. There were between 30 and 40 participants in each module of the BMP workshop, and about 20 of the participants attended the entire workshop. The group included fish farmers, researchers, extension specialists, media specialists, environmental managers, fisheries administrators, and students. About 15% of participants were female. Most participants were from South Africa, but there were individuals from Kenya, Ghana, Tanzania, and Malawi.

Excluding the US and Host Country PIs, there were 38 participants at the conference in Brazil. The group included university researchers and extension specialists, scientists and administrators from governmental fisheries and environmental agencies, feed company representatives, and fish farmers. Fourteen of the participants were female.

### DISCUSSION

The workshops were received quite well by the participants and much discussion was generated. There was a general consensus that BMPs will be widely used in the future through voluntary adoption, government regulations, requirements for loans, and for compliance with water quality standards mandated by governments.

The workshop presented in South Africa and Brazil could be easily presented at other places where ACRSP is involved. The manuscript on BMPs developed for the workshop proved quite useful. It was revised based on the experience gained from using it in the workshop and is attached to this report as Appendix 1.

### CONCLUSIONS

- (1) The BMP workshops presented in South Africa and Brazil were well attended and successful.
- (2) Use in the workshop of the manuscript on guidelines for developing aquaculture BMPs allowed it to be enhanced for future applications.
- (3) The manuscript on aquaculture BMP guidelines should be published by ACRSP as a manual.
- (4) Additional aquaculture BMP workshops could be a beneficial activity for ACRSP.

### ANTICIPATED BENEFITS

The discussions generated by the workshop will no doubt lead to greater awareness of the use of BMPs in aquaculture. In both Brazil and South Africa, the participants expressed the intent to develop national BMPs for aquaculture. The manuscript on guidelines for developing aquaculture BMPs will be published by ACRSP and it can be beneficial to those interested in designing BMPs. There appears to be interest in presenting the workshop at other locations, and the experience gained in the present activity will be greatly beneficial in future BMP workshops.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## AMAZON AQUACULTURE OUTREACH

*Twelfth Work Plan, Sustainable Development and Food Security 1, (12SDF1)  
Final Report*

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## ABSTRACT

Outreach activities have significantly benefited over 129 producers and their families (256 ponds, ~90 ha.) in the Peruvian Amazon (Iquitos-Nauta) and 78 producers (23 females, 37 males, and 18 teenagers) and their families in the Colombian Amazon (Leticia), the latter being in its first year of extension activities. Additionally, the two CRSP-funded extensionists have provided aquaculture training to 68 vocational high school students and Aquaculture Cooperative members (25 females and 43 males) in the Amazon Basin (Brazil, Colombia, Ecuador, and Peru). One of our extensionists (Pedro Ramirez) from Peru was in an exchange program, initiated in the eleventh work plan, in the Ecuadorian Amazon for one month, training a total of 69 (12 females and 57 males) producers in two basic aquaculture-training courses held in El Puyo and Macas. Three international training courses helped provide technical assistance in aquaculture techniques to local and prospective fish farmers. Fifty-seven individuals representing Ecuador, Brazil, Colombia, Venezuela, and Peru participated in the "4<sup>th</sup> International Training Course of Prominent Amazonian Aquaculture Species for Students and Professionals," held in the National University, Leticia (Colombia) Campus from 21–24 July, 2004. Twenty producers and farmers representing Brazil, Colombia, and Peru participated in the "4<sup>th</sup> International Training Course of Prominent Amazonian Aquaculture Species for Producers," held in Leticia from 22–24 July, 2004. Eighteen participants representing Brazil and Colombia attended the "1<sup>st</sup> International Training Course of Ornamental Amazonian Fish Species," held in Leticia from 25–27 July, 2004. The Amazonian aquaculture website, developed in the tenth work plan, is being maintained. This site is an important tool to communicate the work done by research institutions in the USA, many Amazon Basin nations, and elsewhere (over 16,000 hits from Aug. 1, 2004 through Jul. 31, 2006).

## INTRODUCTION

In South America, seven countries have USAID-presence status (Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, and Peru). Excluding Paraguay, these countries are linked by the major river systems of the Amazon and Orinoco Rivers, which contain the largest diversity of freshwater fishes in the world. South America offers a special opportunity to develop appropriate technologies to cultivate alternative aquaculture species native to this continent. The addition of South America to the PD/A CRSP in the eighth work plan has provided considerable and unique opportunities to expand the CRSP Central Database. In the ninth work plan, a prime site was established at Iquitos, Peru, which is in the heart of the Peruvian Amazon (Loreto Region). Three important institutions work with aquaculture in the Peruvian Amazon: Instituto de Investigaciones de la Amazonia Peruana (IIAP), Ministerio de Pesquería (Peruvian Government), and Universidad Nacional de la Amazonia Peruana (UNAP). These organizations have well-designed and equipped aquaculture research stations. *Colossoma* and *Piaractus* are considered by local aquaculturists as the best fishes for commercialization in the tropical part of Peru.

In the eleventh work plan, new sites (Brazil, Colombia, and Ecuador) were added to expand the project to be more inclusive of the Amazon Basin. The major Brazilian Amazon institutions currently conducting aquaculture outreach and research are the Instituto Nacional de Pesquisas da Amazonia (INPA) and Universidad Federal do Amazonas (UFAM). Both institutions work with *Colossoma macropomum* and *matrinxã* or *sabalo* (*Brycon cephalus*). In the Ecuadorian Amazon, the NGO Arcoiris is the leading aquaculture extension institution in the region. They are producing *Colossoma sp.*, *Piaractus sp.*, and *Prochilodus sp.* fingerlings, primarily to stock ponds of small-scale producers and indigenous communities. In the Colombian Amazon, the institutions conducting research in aquaculture are: Universidad Nacional (UNAL), Instituto de Investigacio-

nes de la Amazonia (SINCHI) and Corporación Regional del Amazonas (Corpoamazonia). Colombia fisheries and aquaculture production is 180,440 mt of which 23% was provided by aquaculture (FAO, 2001). The species cultured are: *P. brachyponnus*, *C. macropomum*, *Arapaima gigas*, *Prochilodus sp.*, *Brycon sp.*, *Pseudoplatystoma fasciatum*, and *Cicla ocellaris*. Considerable potential exists to examine other species, as the Amazon Basin is home to over 2,000 freshwater species of fish and innumerable invertebrates.

A Memorandum of Understanding (MOU) is currently in place linking IIAP, INPA, UFAM, UNAL, Arcoiris, Peace Corps Ecuador, and SIUC (and collaborating US universities) into the CRSP network. Facilities have been significantly upgraded/renovated at IIAP during the ninth, tenth, and eleventh work plans. For the twelfth work plan, three projects were funded: 1) Amazon Aquaculture Outreach, 2) Nutrition and nutrient utilization in native Peruvian fishes, and 3) Broodstock Development of Amazonian fishes. These projects are extensions of research and outreach activities first developed during the ninth work plan. Outreach and networking activities have been greatly expanded during the eleventh work plan to facilitate regionalizing the benefits of the CRSP to nearly every country comprising Amazonia.

## MATERIALS AND METHODS

### Objective 1. Provide extension services to the community to promote sustainable aquaculture in the Amazonian region.

One CRSP-funded extensionist continued to reinforce extension activities with fish producers along the road system between the Cities of Iquitos and Nauta, as well as along the

Itaya River (Cahuide and 12 de October communities). The other CRSP-funded extensionist initiated extension activities in the Colombian Amazon (Leticia). All farms were visited on a rotational basis so that every farm was visited at least once each quarter. Farmers were provided with information on fish husbandry for a diversity of novel endemic species (*Arapaima gigas*, *Prochilodus nigricans*, *Brycon erythropterum*, *Osteoglossum biscirasum*, *Cichlasoma amazonarum*- as a forage fish,) in addition to the species that they have traditionally been cultivating (*P. brachypomus* and *C. macropomum*). General pond maintenance, as well as with any new developments learned through research activities, were covered. Standard water quality parameters (temperature, dissolved oxygen, pH, hardness, alkalinity, carbon dioxide, total ammonia nitrogen, and nitrite) and fish weight and length were measured at representative farms throughout the region as part of a follow-up program.

**Objective 2. Provide short courses to governmental and NGO personnel to maintain and expand a network of aquaculture extensionists in the Amazon region.**

Three intensive training courses were offered at the National University, Leticia Campus, Colombia, from 21–27 July, 2004. Fifty-seven individuals participated in the “4<sup>th</sup> International Training Course of Prominent Amazonian Aquaculture Species for Students and Professionals”, from 21–24 July 2004. Twenty producers and farmers participated in the “4<sup>th</sup> International Training Course of Prominent Amazonian Aquaculture Species for Producers”, from 22–24 July 2004. Eighteen participants attended the “1<sup>st</sup> International Training Course of Ornamental Amazonian Fish Species”, from 25–27 July 2004.

**Objective 3. Maintain the specialized website developed in WP10 on Amazonian aquaculture to provide for information exchange and networking.**

A web site (<http://fisheries.siu.edu/amazonia/index.html>) on Amazonian aquaculture and species was expanded to allow for information exchange and networking. This web site contains information on all CRSP-sponsored research and outreach activities in the Amazon region. It also provides links to other agency activities in the region such as USAID, World Wildlife Fund, etc. An “AquaForum” allows for discussions on Amazonian aquaculture and species by interested participants. The web site also contains a specialized bibliography on research and outreach publications related to Amazonian aquaculture and species. An up-to-date list of announcements concerning related workshops and meetings is maintained on the site. A list-serve was established (Domeus) and maintained for the purpose of relaying relevant information on Amazonian aquaculture and species. The number of hits to the site has been enumerated to determine the site’s exposure.

**RESULTS**

**Objective 1. Provide extension services to the community to promote sustainable aquaculture in the Amazonian region.**

Since 1 Aug. 2004, two CRSP/IIAP extensionists conducted monthly visits to 129 producers and their families (256 ponds – 90 ha.) in the Peruvian Amazon (Iquitos-Nauta and the Cahuide and 12 de October communities in the Itaya River) and 78

producers (23 females, 37 males, and 18 teenagers) and their families in the Colombian Amazon (Leticia), the latter being in its first year of extension activities.

Additionally, the two CRSP-funded extensionists have provided one to two day aquaculture training sessions that contained theoretical and/or practical work to 68 vocational high school students and Aquaculture Cooperative members (25 females and 43 males) in the Amazon Basin (Brazil, Colombia, Ecuador or Peru). These training activities included the following institutions:

1. Asociación de Acuicultores de la Región Loreto: Fish Diseases Prevention given to 11 producers (5 females and 6 males); January. 2005, Iquitos, Peru.
2. Technical students: Basic training course on Aquaculture extension techniques in the Amazon region taught by Peruvian extensionist Pedro Ramirez, given to 15 producers (1 female and 14 males); 25–26 February. 2005, Instituto Tecnológico de Sevilla, Macas, Ecuador.
3. Members of the Sarayaku Indigenous community: Course in pond construction and management taught by Peruvian extensionist Pedro Ramirez, given to 54 producers (11 females and 43 males); 5 March 2005.
4. 1<sup>st</sup> International Amazonian Fish Culture Fair. CRSP Colombian extensionist (Gabriel Barreto) participated in the organization of this fair, which had almost 7,000 visitors and participants from Colombia, Brazil and Peru. Leticia, Colombia; 21–23 March 2005.
5. Centro Educativo Primario Secundario Mixto Quistococha 60110, Institución Educativa Primario Secundario Mixto Cruz del Sur 60128 and Institución Educativa Primario Secundario Peña negra 601608: Fish Culture Course given to 28 students (8 females and 20 males); 15–16 July 2005, Iquitos, Peru.
6. Producers Iquito-Nauta Road: 1st course on Basic Aspects of Aquaculture Economics, given to 9 producers (6 females and 3 males); 28–29 Oct. 2005, Iquitos, Peru.
7. Producers Iquito-Nauta Road: Training Course on Fish Nutrition and Feeding, given to 27 producers (10 females and 17 males); 25–26 Nov. 2005, Iquitos, Peru.
8. Producers from the Cahuide community in the Itaya River: Training course on fish cage culture and feeding, given to 11 producers (3 females and 8 males); 28–29 January 2006.
9. Producers from the 12 de Octubre community in the Itaya River: Training course on fish cage culture and feeding, given to 13 producers (3 females and 10 males); 27–28 March 2006.
10. 2<sup>nd</sup> International Amazonian Fishculture Fair. CRSP Colombian extensionist (Gabriel Barreto) participated in the organization of this fair, which had nearly 8,000 visitors and participants from Colombia, Brazil and Peru; Tabatinga, Brazil, 12–14 April 2006.

**Objective 2. Provide short courses to governmental and NGO personnel to maintain and expand a network of aquaculture extensionists in the Amazon region.**

Three intensive training courses were offered at the National University, Leticia (Colombia) Campus, from 21–27 July 2004, for governmental, non-governmental personnel, professionals, indigenous communities, small-scale producers and students conducting aquaculture activities in the Amazon Basin. The first course (4<sup>th</sup> International Training Course of Prominent Amazonian Aquaculture Species for Students and Professionals) was held from 21–24 July 2004, with 57 participants representing Ecuador,

Brazil, Colombia, Venezuela, and Peru. The second course (4<sup>th</sup> International Training Course of Prominent Amazonian Aquaculture Species for Producers" was held from 22–24 July 2004, with 20 producers and farmers representing Colombia, Peru and Brazil. The third training course (1<sup>st</sup> International Training Course of Ornamental Amazonian Fish Species) was held from 25–27 July 2004, with 18 participants from Colombia, Peru, and Brazil. These intensive training courses, consisting of two- to three-day lectures and one day of practical laboratory work, trained potential producers and apprentice aquaculturists on the production of some prominent native species. The participants learned pond construction, broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, and disease prevention and treatment, all specifically related to native cultured species such as *Colossoma sp.*, *Piaractus sp.*, *Arapaima sp.*, *Prochilodus sp.* and *Brycon sp.* (fish), and mollusks (Congompe and Chur). In addition, they learned techniques to teach hormone injection, spawning, fertilization, incubation and larviculture. A CD-ROM displaying all the course material for the Amazon aquaculture-training course was produced.

The following students conducted practical training on extension techniques at the Quistococha UNAP research and training facility (Iquitos, Peru):

1. German Gonzalez Aspajo (student from UNAP, Iquitos). Fish monitoring techniques for producers de la carretera Iquitos-Nauta for 1 month (September 2004)
2. Guillermo Yatto Rios (Student from Puerto Maldonado Technical School). Capacitacion en acuicultura. 20 days (November– December 2004)

**Objective 3. Maintain the specialized website developed in WP10 on Amazonian aquaculture to provide for information exchange and networking.**

Amazon Aqua Forum (Domeus), added in July 2003 to the Amazon website, allows users to formulate questions to other users. From August 1, 2004 through July 31, 2006, there were 16,044 hits (SurfstatsV.6.0, 2000). Based on registered Domain names and highest registered hits, the following countries have visited the site: Switzerland, Brazil, Sweden, Peru, Colombia, Germany, U.K., Portugal, Spain, Norway, Austria, Taiwan, Mexico, USA, Belgium, Japan, Chile, Australia, Argentina, Canada, and Hungary.

**DISCUSSION**

The two CRSP-funded extensionists provided considerable technical assistance to inhabitants of the Amazon Basin, particularly in Colombia, Ecuador and Peru. Several bilingual indigenous teachers were trained to enable them to provide ongoing assistance to ensure sustainable aquaculture development beyond the life of the aquaculture CRSP. The International training courses gave continuity to the channels initially opened by CRSP in the tenth and eleventh work plans for the creation and support of a network of aquaculturists in the Amazon Basin. Some of these aquaculturists gained expertise to more fully function in extension or production activities. Finally, the expanded website received an ample array of visitors from countries in the Amazon region and throughout the world. This website was used by researchers to exchange questions and answers with CRSP researchers pertaining to the aquaculture of native species in the Amazon region and elsewhere.

**CONCLUSIONS**

The extension services provided to aquaculture producers of the Amazon Basin have been highly beneficial. By training a number of bilingual indigenous teachers to provide continuous aquaculture extension, we have ensured these benefit will continue to accrue, at least at a modest level, well beyond the life of the Aquaculture CRSP. The International training courses gave continuity to the channels initially opened by CRSP in the tenth and eleventh work plans for the creation and support of a network of aquaculturists in the Amazon Basin. The website has become an important tool to communicate aquaculture research being conducted by institutions in the USA, Amazon nations, and elsewhere.

**BENEFITS**

Aquaculture is an alternative form of agriculture offering significant benefits to rural residents and farmers throughout the Amazon Region. Aquaculture requires considerably less land than needed for cattle ranching. Ponds can be used year after year whereas rainforest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons. Aquaculture will benefit both rural and urban poor through the addition of a steady supply of high quality protein in the marketplace.

Studies in the twelfth work plan investigated the nutrition and nutrient utilization in native Peruvian fishes and the key nutritional requirements and ecological significance of several Amazonian freshwater fish species, particularly *C. macropomum* and *P. brachypomus*, to improve and develop sustainable aquaculture technology. Techniques learned through research activities were subsequently taught via extension activities to producers and other aquaculture extensionists/educators. The development of sustainable aquaculture will benefit many sectors throughout the Amazon region. Rural farmers and indigenous communities benefited 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 benefited 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 two frugivorous fishes have been suggested to play a crucial ecological role in disseminating seeds from the flooded forest (Goulding, 1980; Araujo-Lima and Gouling, 1997). Accordingly, the aquaculture of *Colossoma* and *Piaractus* may be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Amazon region. 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 benefited 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.



This study contributed to capacity strengthening by providing training for IIAP staff on various aspects of fish nutrition and reproduction. Results of the training include:

1. 129 producers and their families (256 ponds – 90 ha) from a diversity of ethnic groups received extension services in the Peruvian Amazon.
2. 78 producers (23 females, 37 males and 18 teenagers) and their families from a diversity of ethnic groups received extension services in the Colombian Amazon (Leticia).
3. 68 vocational high school students and Aquaculture Cooperative members (25 females and 43 males) were trained in basic aquaculture concepts by the two CRSP-funded extensionists in the Amazon Basin (Brazil, Colombia and Peru).
4. 69 (12 females and 57 males) producers from El Puyo and Macas (Ecuador), were trained in two basic aquaculture courses by one of the CRSP extensionists (Pedro Ramirez) from Peru who participated in an exchange program, initiated in the eleventh work plan.
5. 5 countries from the Amazon Basin received direct benefits through the training of participating students, professionals and farmers.
6. 16,044 hits from 29 countries and groups occurred on the Amazonian aquaculture website.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## UNDERSTANDING THE AGRICULTURAL KNOWLEDGE SYSTEM FOR THE DEVELOPMENT OF AQUACULTURE IN NICARAGUA: ECONOMICS, MARKETS, AND INSTITUTIONS

*Eleventh Work Plan, Sustainable Development and Food Security 2 (12SDF2)  
Final Report*

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### ABSTRACT

The flow of information between researchers, extension agents, educational institutions, and producers involved in tilapia culture should produce technologies that better suit the needs of producers. Understanding the aquaculture knowledge system requires the identification of stakeholders, their interactions, and the documentation of producers' knowledge. In Nicaragua, the analysis of the Aquaculture Knowledge and Information System (AKIS) for tilapia culture revealed that the system was not well developed. Tilapia culture was a highly subsidized activity undertaken by only a small number of producers mainly located in Northern Nicaragua. The level of producer knowledge was low and ignored by other stakeholders in tilapia culture. Researchers, extension agents, and educational institutions did not often shared nor discussed their experiences in tilapia culture with each other; thus, each institution worked largely in isolation. The analysis of the AKIS also showed that small and medium scale tilapia culture had not developed due to a pattern of government support that has favored shrimp production over fish culture, lack of domestic supply of good quality commercial feed and fingerlings, and the environmental controversy over large-scale tilapia culture in Lake Nicaragua. The future of small and medium scale tilapia culture depends on the successful expansion of export oriented tilapia culture that could attract other business such as input suppliers.

### INTRODUCTION

The decision-making processes of aquaculture producers are dynamic and unique to each situation (Warren, 1991; Warren and Rajasekaran, 1993). Documenting that decision process is crucial to further develop aquaculture because it answers the following question: how do producers try to solve their problems using their own knowledge? (Röling, 1988; Rajasekaran, 1993). Being aware of the importance of farmers' knowledge, in 2000, the Food and Agriculture Organization (FAO) and the World Bank issued a document proposing the integration of farmers, educational, research, and extension institutions into an Agricultural Knowledge and Information System for rural development (AKIS) (FAO and World Bank, 2000). This new perspective was intended to respond to the knowledge and information needs of large numbers of rural communities by helping them reach informed decisions on the better management of their farms, households, and communities (FAO and World Bank, 2000).

For the purpose of this study, FAO and World Bank's AKIS framework was adapted to aquaculture as the Aquaculture Knowledge and Information System. The application of the AKIS approach to aquaculture was necessary to perform an analysis of the level of collaboration among producers, researchers, extension agents, and educational institutions

involved in tilapia culture in Nicaragua. The adjustment of FAO and World Bank's AKIS to aquaculture in Nicaragua seemed quite logic and proper. According to Veverica and Molnar (1997:399) "In general, extension approaches and notions developed for land-based agriculture are applicable to aquaculture." However, the authors stressed the importance of two main differences between aquaculture and agriculture; aquaculture is rather a new activity in many regions, and it employs extension agencies units that often are weakly connected to agriculture extension. The authors continued their argument declaring, "aquaculture can be considered in the context of a new, nontraditional crop."

### AQUACULTURE IN NICARAGUA: AN OVERVIEW

The first important effort to promote and develop aquaculture in Nicaragua was an initiative by the Sandinista government in 1982. The program started with the creation of the Fisheries National Institute (INPESCA) and the construction of the first aquaculture farm in Nicaragua (FAO, 1992). The mission of INPESCA was to promote tilapia (*Oreochromis* sp.) and carp (*Cyprinus* sp.) culture as a means for improving the diets of Nicaraguans, as well as marine shrimp as an export oriented crop.

The project executed by INPESCA did not achieve the expected results (FAO, 1984). By 1984, the project was already failing due to lack of properly trained personnel at different levels, inappropriate infrastructure, low quality broodstock, inappropriate technical equipment, limited technical assistance, poor organization, and lack of funds (FAO, 1989). Today, it is clear that the apparent successful promotion and adoption of tilapia culture during the period 1982–1984 was an illusion. If people were building small ponds and producing tilapia, it was because INPESCA was providing fingerlings at no cost. Potential adopters, with little technical knowledge, were willing to “try” the new technology as a subsidized novelty. Once the subsidy was reduced and even eliminated, producers abandoned the activity.

In 1989, the apparently significant adoption of fish culture was already at a halt; tilapia production in rural areas was not growing at all (FAO, 1989). The remaining producers were members of cooperatives that obtained most of their inputs through government agencies. By 1992 the government had stopped all activities in fish culture.

### Recent Efforts to Develop Pond Culture

In 2000, a new project started in the poor northern region. The main objective of the project was to provide a source of animal protein to the dwellers of several rural communities. The first phase of the project was a coordinated effort by the international organization CARE and the government agency Rural Development Institute (IDR). The IDR functioned as the administrator of the funds provided by the Inter-American Development Bank (IDB), while CARE executed the project as part of their broad project RENACER (Natural Resources, Training, and Rural Economics) (Saavedra et al., 2003).

The project included a total of 56 ponds from which, 37 were located in the municipality of Pueblo Nuevo and 19 in the municipality of Totogalpa. The project’s approach included the adoption of a production system based on the use of livestock manure to fertilize ponds, locally available feedstuffs, pond-fertilized water to irrigate adjacent crops, and household consumption of 20% of the production to ensure food security, while the rest of the production would be sold to generate income (Saavedra et al., 2003). Despite common issues in the two communities, the project ceased its support for tilapia production in Totogalpa, while it extended its efforts in Pueblo Nuevo where water supply was less of a problem.

Meanwhile, some individuals and small institutions were carrying isolated tilapia culture projects in several communities. In 2002, a total area of 2.3 ha, including floating cages (2% of total area), was under tilapia production in the departments of Managua, Masaya, Granada, Matagalpa, Jinotega, Estelí, and Madriz. The producers consumed most of the production of those projects, even though sales of tilapia to neighbors were occurring. The main issue for the small producers in those projects was the need to improve and increase the size of the ponds, because low production capacity and poor pond construction were considered as main barriers to profitability (Saavedra et al., 2003).

The aquaculture sector in Nicaragua began in 1982. Ever since, many producers, extension agents, donors, researchers, and educational institutions have interacted to develop tilapia culture; however, their degree of success has been modest. A description of the Nicaraguan AKIS should provide informa-

tion about the different capacities, advantages, interactions, and roles of current stakeholders.

### Problem Statement

The main objective of this paper is to understand the AKIS for tilapia culture in Nicaragua. To achieve that broader goal, it is necessary to accomplish three specific objectives:

1. To conduct a stakeholder analysis (SA).
2. To assess producers technical knowledge, and
3. To conduct an analysis of strengths, weaknesses, opportunities, and threats (SWOT) as perceived by producers.

### METHODS

The sampling for this study started when several tilapia producers were approached during a short seminar on tilapia culture and pond construction held by an ACRSP team in Estelí, Nicaragua from November 9–12, 2005. The researcher requested to the seminar attendees their participation and collaboration. Those who assisted in the short seminar, and offered to participate led to other stakeholders in their areas.

The sampling technique used to identify potential respondents is called network snowball sampling. Snowball sampling is used when there is an interest in sampling an interconnected network of people and organizations where each is connected with another through direct or indirect relationships (Neuman, 1997). Two types of interviews were used. A total of 13 open-ended interviews were conducted with producers, whereas seven semi-structured interviews were used with other stakeholders.

### RESULTS

#### Stakeholder Analysis

The identified primary stakeholders in tilapia culture in Nicaragua were the producers. At the time of the study 90% of the producers were members of the cooperative COOSEM-PROTIR, R.L. Their farms were located at the communities of Pueblo Nuevo, Los Horcones, and San Juan de Limay. The members of the cooperative had a total of 20 ponds with a total area of 5,375 m<sup>2</sup>. Five producers had only one pond, four had two ponds, one had three ponds, and one had four ponds. The average pond size was 343 m<sup>2</sup> with an average depth of 1.5 m. Four producers built small ponds to nurture the fingerlings before putting them in the larger pond; the nursery ponds averaged 53 m<sup>2</sup>.

One of the three identified producers that work alone was not interviewed because his farm was located far from the others. The two interviewed producers were working in relative isolation from the other producers. One was operating six km out of the city of Ocotal. He stocked 3,000 20-g fingerlings in 200 m<sup>2</sup> ponds built with brick and covered with cement. His source of water was a creek, and he bought fingerlings from the UNA and commercial feed from one national supplier.

The other producer was located in Granada. He owned one pond of 165 m<sup>2</sup>, three of 22 m<sup>2</sup>, two of 6 m<sup>2</sup>, and one of 12 m<sup>2</sup>. The ponds were constructed to use the walls of a large water tank. For that reason, several of the ponds were constructed inappropriately; they only received sunlight half of the day since the taller tank obstructs the rays. This producer bought 3,000

fingerlings from the National Agrarian University (UNA) to stock the ponds and commercial feed from a national supplier. He had not yet harvested a crop.

The first identified secondary stakeholder was the IDR. At the time of the study the IDR represented the main secondary stakeholders in tilapia culture in Nicaragua and the main source of subsidy for the industry. Its activities in Estelí were known by most stakeholders in tilapia culture, and were perceived as a success story. The activities of IDR were the second phase of a project initiated by CARE-RENACER in 2000. The project had a total cost of US\$135,469, with US\$105,999 financed by the Inter-American Development Bank through the IDR. The producers in the project financed US\$29,469 through bank loans, and sales of cattle and fish. The objectives of this tilapia project was to reach a stable production of 2,270 kg per month for the national market with 35 producers, and to build at the end of the project a US\$20,000 fingerlings production farm in the area.

In addition to providing technical assistance, IDR personnel bought all the inputs needed by the project. IDR personnel also helped market the product by providing coolers to transport fish and accompanying fish producers to fairs in several cities in the area. According to several producers, the IDR should help with the marketing of the product because they promised that all the production in the project would be sold to an exporting company at a high price.

UNA was another secondary stakeholders. It works as an education and extension agent. It is involve in tilapia culture through the "Granja Demostrativa de Cultivo de Peces." This demonstration farm was built in 1982 but its operation was interrupted in 1984. Since 1999, it has operated under the combined effort of the UNA and ADPESCA. The main objectives of the farm are fingerlings production, technical training for potential producers, and laboratory for students. It promotes tilapia culture by distributing free fingerlings.

### UCA

UCA operates as a research, extension, and educational center. It has been involved in tilapia culture since the construction of its tilapia farm in 1982. The farm has the capacity to produce 16,000 fingerlings per time cycle. The main objective of the tilapia farm is fingerlings production for its own grow-out farm. The grow-out is done under integrated aquaculture, where the fertilized water from the ponds is also used to irrigate crops. UCA has been negotiating with a private company to supply fingerlings and technical assistance to an export-oriented project using cage production.

### ADPESCA

The function of ADPESCA includes analyzing aquaculture projects, keeping record of the number of farms in operation, issuing technical guarantees, and evaluating the environmental impact of tilapia projects. ADPESCA publishes the *Anuario Pesquero y Acuicola de Nicaragua*. This yearly publication contains data on marine fisheries and wild-caught and aquaculture shrimp. The information for tilapia is minimal, since no national plan for the development of the activity exists.

### UCATSE

Mainly involved in research and education, UCATSE has its own Agro-aquaculture station "Los Chilamates." Since 2002, however, the station has been underutilized due to lack of funds. The station was in need of new broodstock and laboratory equipment to carry out basic analyses. The research conducted by students in UCATSE was oriented to testing the viability of fingerling production in different parts of the year and to use cheaper substitutes for traditional inputs in fingerlings production. However, since 2003 no additional studies have been conducted because the farm is used mainly for production purposes.

### Pan-American Agriculture School "Zamorano."

This institution is an extension, research, and educational center located in Honduras, approximately 70 kilometers from the border between Honduras and Nicaragua. The person on charge of the aquaculture section and head of aquaculture in "Zamorano", Dr. Daniel Mayer is considered an authority in tilapia culture in the region. He has trained several Nicaraguan producers and technical personnel. In fact, a manual elaborated by Dr. Meyer, has been used as a guideline for tilapia culture by researchers and educators in Nicaragua. Lately, "Zamorano" has become the new supplier of fingerlings for the IDR project.

### CARITAS

The Catholic non-government organization has just recently been promoting tilapia culture as a minor component of their activities. Its extension agents tend to have very basic knowledge of tilapia production. CARITAS could play an important role promoting small-scale tilapia culture among the more than 3,000 producers participating in its project. The main objective of the CARITAS is to improve the economic situation of its more than 3,000 beneficiaries through the transfer of new technologies. Those technologies have focused on vegetables production, the use of byproducts, and use of water.

### CIRA

Finally, the last secondary stakeholder was the CIRA, an entity of the Universidad Nacional Autónoma de Nicaragua (UNAN). The center is a research institution that has legal authority over the quality of water in Nicaragua. The CIRA has played a very important role in the negative campaign against tilapia culture in Nicaragua. Its studies have been used by environmentalist to justify a campaign against the operation of large-scale farms in the Managua and Nicaragua Lakes.

### Stakeholders' Importance and Influence

**Primary Stakeholders:** The primary stakeholders, the producers, did not have any influence on tilapia culture in Nicaragua. Their total number was too small to have any political power and their budgets did not allow them to fund lobbying activities to advance their cause.

**Secondary Stakeholders:** None of the secondary stakeholders would be affected if small and medium scale tilapia culture ceases. All of them carry out other programs; thus, tilapia culture was just one of many. Regarding stakeholders influence, of all

the secondary stakeholders, only the CIRA has a sufficient influence to affect the future of small and medium scale tilapia culture. Its political power could be used to undermine any effort to develop small and medium scale tilapia culture as it has demonstrated in its efforts to terminate large-scale tilapia culture.

### Producers' Empirical Knowledge

The level of empirical knowledge among tilapia producers in Nicaragua is very low in general. Producers have not accumulated much experience, conducted informal experiments, nor understood the place of tilapia culture in their environment. Tilapia culture among current producers is an innovation recently introduced as an alternative to traditional crops (Saavedra et al., 2003).

The experience with tilapia culture is recent. It started in 2000 and has yet to be passed over generations. The management practices are yet being modified; testing and innovativeness are very limited. Most producers were just learning the basics of tilapia culture. In most cases, they relied heavily on IDR personnel for the management of their ponds. The records gathered during the interviews showed that even producers with six years of experience relied almost totally on the recommendations provided by the IDR.

### Description of the Production Process

At the time of the study, ponds were built using manual labor, in mainly sandy or clay soils. For that reason, it was necessary to cover the inside of the ponds with plastic. Once the pond was stocked, producers kept the water level and fertility of the pond by adding water and more manure, according to the standards established by the technician. However, several producers did not always comply with the technician's instructions because they did not want to spend too much time pumping water. Others, with easier access to water, practiced water exchange more often and used the fertilized water to irrigate crops planted around the pond.

Another practice was fish sampling. Starting 15 days after the pond was stocked, sampling continued until the seventh month of the production cycle. Sampling was performed with help of the technician and consisted of extracting 50 tilapias, which were weighed and returned to the pond. Then, the technician used the information to adjust the feeding recommendations and determine if the fish were ready to be sold. Despite the apparent utility of sampling, several producers expressed that they did not like to do it because some fish were lost in the process. On average, harvest started after seven months, and continued for three more months. Producers harvested their ponds to feed their families, share with relatives and friends, sell to neighbors, and in some cases, sell to an intermediary. Once the pond was empty, producers cleaned it, applied lime, made repairs, and prepared it for the next crop.

### SWOT Analysis

The analysis of strengths, weaknesses, opportunities and threats provided information about the individual opinions of producers regarding their farms and the surrounding environment. The perceptions about the farm (internal setting) were depicted by the strengths and weakness.

### Strengths and Weaknesses

The producers mentioned the following strengths: The analysis of strengths and weaknesses indicated producers perceived water access (easy or difficult) and location (convenient or inconvenient) as the most relevant internal characteristic of their farms. Those findings confirmed the disparity of conditions faced by the members of the cooperative in Pueblo Nuevo and Los Horcones. Some farms had easy access to water, but were set in an inconvenient location (located in the high part of the watershed). On the other hand, other farms had difficult access to water, but were set in a convenient location (located in the low part of the watershed). Among farms with difficult access to water it was necessary to use electric or gas pumps to fill the ponds. In contrast, in farms with easy access, water was transported by gravity.

### Opportunities and Threats

The analysis of opportunities and threats gives an idea about consumers' expectations and concerns on the effect that external factors could have on their farms. The analysis of external factors revealed that producers expected that opportunities would come as a result of growth in the export and tourism markets. The threats expected by producers could be related to the reduction of water access in the area due to deforestation of the watershed. Higher water costs due to a new legislation may tax water collection from creek, springs, and underground streams. The possibility that the IDR would disengage from the project, and the risk that the fingerling farm would not be built were other concerns. Without the IDR, it would be very difficult to have easy and cheap access to commercial feed and fingerlings.

### CONCLUSIONS

At the time of the study, small and medium scale tilapia culture in Nicaragua was a highly subsidized, minor economic activity carried out only by few producers. The stakeholders of small and medium scale tilapia culture did not form a well-integrated knowledge system. Each secondary stakeholder worked in isolation and had particular interests. As a result, the needs of producers were not addressed nor considered to further develop tilapia culture. Tilapia culture was delivered as an outside technology that small producers could not afford without help.

In general terms, the development of small and medium scale tilapia culture in Nicaragua was limited by five factors: a flawed development approach by the government, lack of good quality inputs, competition for public funds with the shrimp culture, the campaign of environmentalists, and the lack of an integrated knowledge system.

Tilapia culture has lost government support gradually since the middle of the 1980s. The fact that tilapia production was to supply the domestic market and shrimp culture was focused on the export market made a significant difference; shrimp exports were generating foreign currency and tilapia culture was not. That made government officials focus its limited funds on shrimp culture rather than tilapia culture. After INPESCA, subsequent government agencies had withdrawn even more from tilapia culture.

Although environmentalists oppose the operation of large-scale farms, the campaign against tilapia culture in the lakes

has affected small and medium scale producers of tilapia culture the most. This campaign has partially been based on blaming tilapia culture for the pollution of the lakes. Thus, if the lakes were polluted, tilapia coming out of them would be polluted as well. For that reason, consumers fear eating tilapia, no matter the origin, wild-caught or farmed, even if they are willing to consume it, especially those with lower incomes, they are only willing to pay a low price, generally the price for wild-caught tilapia.

Marketing whole farmed-tilapia for a profit is very difficult for two reasons. On one hand, wild-caught tilapia from the lakes and reservoirs are relatively inexpensive. On the other hand, many fish consumers believed that nearly all tilapia came from Lake Managua and, therefore, it is contaminated in some way. Consumers' rejection of tilapia stemmed mainly from extended campaigns executed by environmentalist groups that opposed tilapia culture and favor tourism projects in lakes Managua and Nicaragua.

The members of the cooperative sold some tilapia in their area because people know how the fish were produced and because other fish species from distant lakes and the ocean were available at higher prices. But those markets were small, and could not support the development of profitable tilapia culture in the region. As a producer expressed, "I like tilapia culture, but to really work on it, I have to have at least three or four ponds; for that, I need a larger market." That is important, because in order to develop rural aquaculture, producers need to have access to the larger markets in the country.

The results indicated that the development of small and medium scale tilapia culture is uncertain, but as one stakeholder expressed, "Tilapia culture at all levels will develop, but it will follow the shrimp industry model; large farms will attract input suppliers and grow, and as the large farms grow, small and medium scale producers will have access to inputs and will sell their production to the exporters during the peak season, and will supply the domestic market the rest of the year." Further studies to develop small and medium scale tilapia culture should focus on answering several questions. Can all tilapia culture stakeholders come together and form a system? Can the potential producers obtain technical assistance, inputs, and a market on their own? Can they afford to pay for technical assistance and buy inputs without a subsidy? And, finally, do they have the ability and the means to market their own production?

Future interventions in Nicaragua should start by assembling stakeholders and beginning a rational dialogue on the industry. Nicaragua has all the elements to make of tilapia culture an important economic activity; it has research and educational institutions with highly trained personnel on aquaculture and the nation has abundant water resources for fish culture. However, tilapia culture has been promoted while ignoring local conditions and using inappropriate approaches.

#### Anticipated Benefits

The resulting study could provide relevant information of the AKIS in Nicaragua, which could be used by national and international organizations interested in the further development of aquaculture in Nicaragua. According to Rivera et al. (2006:21), "An [AKIS] assessment will reflect the needs of the specific country and the specific context and stage of development in which it operates, address the institutional constraints and opportunities

inherent in the country as a whole, clarify the extent to which it is institutionally pluralistic, and identify where are the strengths and weaknesses of its knowledge system."

The Aquaculture Collaborative Research Support Program (ACRSP), the program providing the funds for this study, makes information on diverse aquaculture topics available to farmers, educators, other researchers, public policy makers, loan officers, and investors (Veverica & Molnar 1997). The information provided is the result of a series of research activities funded by the ACRSP, and oriented to develop tilapia culture in Nicaragua as part of a larger focus on Central America. This study will provide new information that can be used by the ACRSP and other individuals and institutions to orient future training and research activities in regard to further develop tilapia culture in Nicaragua.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## ASSESSING THE POTENTIAL FOR AQUACULTURAL DEVELOPMENT TO PROMOTE FOOD SECURITY AMONG INDIGENOUS PEOPLE IN GUATEMALA

*Eleventh Work Plan, Pond Design and Watershed Analyses Training 3 (12SDF3)  
Final Report*

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### ABSTRACT

Guatemala is a multi-ethnic, multilingual and highly stratified society in which 55% of its 14.7 million people are indigenous Maya, Xinca, and Garifuna peoples. The people of non-European origin are much poorer and reside in more remote and difficult locations than their wealthier Ladino minority. Freshwater ponds total about 100 ha, an area less than 10% of the total surface dedicated to shrimp production. Some additional 26 ha produce freshwater prawns for domestic consumption.

In 1989, FAO reported that five tilapia species (*T. mossambicus*, *T. rendalli*, *O. niloticus*, *O. aureus*, and *S. hornorum*) have been introduced and stocked in ponds, in large water bodies, or released in open watersheds. The Peace Corps and governmental technical assistance constructed nearly 600 small ponds in recent decades. Most ponds are managed on subsistence and semi-commercial levels, but the coffee crisis and growing market potential has increased interest in tilapia production. Fish are harvested for home consumption and surpluses are sold in local markets. Women often are responsible for the daily management and feeding of ponds while men are responsible for pond construction and harvest.

Two case study areas were chosen where several indigenous communities have sustained involvement in tilapia culture over extended periods. These communities are located at moderate altitudes in Central Guatemala in coffee-growing areas. Although ponds are small, communal interest in tilapia production is sustained by the absence of alternative sources of fish. The study examines prospects for small and medium-scale tilapia culture in Chimaltenango and Quetzaltenango—locales where the need is great, market access is often limited, and conditions are often suboptimal for production. The guiding questions center on understanding the motivating interests, barriers, and appropriate intervention points for aquacultural development in indigenous communities.

The communities that practice fish culture seem to take pride in communal accomplishment in developing the pond site and rearing repeated crops of fish. Economic returns were not estimated by this study, and are unlikely to be competitive with the cost of the capital employed. The amount of fish produced, even if sold at prevailing prices would not produce a return sufficiently motivating to most producers. Nonetheless, the enthusiasm and pride that residents expressed about their fish ponds reflects the community development achievements represented by the pond. The community achievement represented by a functioning pond with periodically yields was sufficiently motivating for the La Bendición community to build an additional pond. Despite the pond's poor construction, lack of gravity flow water, and the disappointment stemming from the ill-functioning pump, participants were eager to find another way to build additional ponds in more appropriate locations. While lauding the enthusiasm exhibited in these communities, nongovernmental organizations and other advising indigenous rural communities in Guatemala should carefully address site selection and the community context for tilapia culture. Pumping costs are not typically part of an economically sustainable approach to tilapia culture in cool water areas. Water supplies should be sufficiently malleable and reliable to enable producers to fill and replenish ponds whenever water is needed. Thus, local springs, streams, and irrigation schemes represent possible sources of water.

## INTRODUCTION

Indigenous peoples are at the cutting edge of the crisis in sustainable development. Their communities are concrete examples of sustainable societies, historically evolved in diverse ecosystems. For the poorest Guatemalans, food security and sustainable livelihoods remain a continuing struggle among the descendents of the Mayans and other peoples (Smith, 1984). For families in communities in locales with the appropriate conditions, tilapia culture can be one means for providing income, increasing food security, and meeting critical nutritional needs. Some efforts have been made to support the diffusion of fish farming in Guatemala (Lovshin et al., 2000), yet the growth of the enterprise across Central America suggests that efforts to extend fish culture to indigenous people in rural Guatemala may be particularly rewarding.

Guatemala is a multi-ethnic, multilingual and highly stratified society in which 55 percent of its 14.7 million people are indigenous Maya, Xinca and Garifuna peoples. In some areas of the country, particularly in the rural areas, indigenous peoples constitute the vast majority of the population (Stavengren, 2002). Guatemala's highland region, where most of the indigenous population lives, cuts across the country from west to east. The rugged main range includes the highest point in Central America (4,211 m) and flanked on the Pacific side by a string of volcanoes (some active). Volcanic eruptions, floods, and hurricanes have plagued Guatemala throughout history. In the center of the range is Lake Atitlán, where some cage culture of tilapia is currently underway. South of the highlands is the Pacific coastal lowland where large-scale aquaculture is beginning. New fish farms are being established as well as in the Caribbean lowland beyond the tropical forest Petén. Early lessons with credit have shown that the absorptive capacity of indigenous communities is generally low. This is partly due to lack of experience with credit, and partly due to the fact that most of these economies are at the subsistence level or are marginally monetized. Consequently, farmers become indebted and worse off than they were before (IFAD, 2004). Efforts to extend tilapia farming to indigenous communities would take sober guidance from these experiences.

IFAD (2002) notes the importance of strengthening existing organizations and traditional governance systems for sustainable development. A project in the Cuchumatanes Highlands helped existing community organizations – virtually inactive during the 36-year armed conflict – to consolidate relations among members and provided them with need-based training. It is such projects that have already created participatory organizations of indigenous people that should be an initial target clientele for training and technical assistance in tilapia culture (IFAD, 1998; 2001).

In Guatemala, altitude usually determines temperature and is a principal parameter shaping the viability of tilapia as a farm enterprise. In the highlands the daytime air temperatures average between 20°C and 25°C all year long, but can fall below 10°C in December to February. At the highest altitudes, frosts can happen any day of the year and snow can fall even in summer. Indigenous communities with good rainfall and potential pond water temperatures around 22°C might be considered an initial target category of communities for investigation. These roughly correspond to the coffee-growing belt in mountain areas less than 2000 m in elevation.

In Guatemala, the total of freshwater ponds just exceeds 100 ha, which is less than 10 percent of the total surface dedicated to shrimp production. Some additional 26 ha produce freshwater prawns for domestic consumption. APT (2004) reports an example of tilapia as a rotational crop in coastal shrimp ponds in Guatemala. Salinity tolerant hybrids were imported from Israel and introduced into brackish water shrimp ponds. Zubieta (1999) notes a great need for technical assistance and training projects, including the introduction of new farm enterprises, for indigenous communities. He emphasized projects that have potential for selling products in order to increase the community's income and reduce food insecurity, as well as searching for alternative financial resources for the projects, and credits for seeds and fertilizer. Tilapia culture could provide viable opportunity for some indigenous communities (Castillo et al., 1992).

Although it is essential to provide more technical assistance and self-management training for these communities to overcome poverty, sustainable projects must also prevent dependence and paternalism. Moreover, sensitivity to cultural issues and language is an essential ingredient (IITC, 2002). Clearly, consultation with Peace Corps records and knowledgeable personnel will be a necessary step because they have experience working in these locales.

An integrated fishpond project targeted poor farmers that had an average land holding of 0.9 ha per household and an average total annual income of US\$700. By 1989, 1,200 ponds had been built or renovated. About 15 percent of these ponds were integrated with animals and 21 percent with vegetable gardens (Lovshin, 1999). Almost half of the pond operators revealed that a strong motive for retaining an active fish pond was the need for water during the dry season for irrigation and livestock watering (Lovshin, 1999). Most farmers had their irrigated gardens on land that received water by government controlled irrigation canals. Water was rationed during the dry season and farmers had permission to receive water once every two to three weeks. Thus, fish ponds were filled to capacity when water was available and water was dispensed as needed over the period when irrigation canal water was unavailable. Without the fish pond, vegetable production would be impossible or restricted during the dry season (Lovshin, 1999).

A main source of technical assistance for tilapia in Guatemala is the Marine and Aquaculture Study Center of the University of San Carlos (USC). This institution offers graduate studies in the field of aquaculture and training courses at the intermediate level; 34 technicians have been trained to-date. Zamorano personnel have taught in USC training programs. Extension agents have also received on-the-job training during participation in technical assistance projects. Inorganic (chemical) fertilizers, another input used in intensive fish and shrimp farming, are readily available in the larger countries of the region, and to a lesser extent also in the smaller ones, although more costly as they have to be imported (Noriega-Curtis and Rivas, 1989). In Guatemala, the Fisheries and Aquaculture Directorate, formerly by US Peace Corps, CARE, and by a Chinese technical mission, provide aquaculture extension. Extension is oriented toward the transfer of technology and the training of small-scale fish farmers but institutional biases and unrest have left indigenous people out of many of these programs. Whenever possible, extension programs also promote integrated agro-aquaculture practices as a means of improving the utilization of the resources available to small farms (Noriega-Curtis and Rivas, 1989).



Table 1. Selected Sources of Technical Assistance in Fish Culture in Guatemala, 2005

NGOs and Institutions	Type of Assistance
CAESA (NGO), supported by Guatemala Government	Support poor people in Jalapa
CARE of Guatemala	Broad set of projects
Christian Church of Chiquimula	Support poor people of Ocotán and Camotán
Kabawil Peasant Council	Assist farmers in land reform and support of alternative enterprises, such as fish culture
Marine Culture and Aquaculture Center (CEMA) of San Carlos University	Training, advisory services, fingerling production and distribution
Movimundo, Italian NGO	Assist poor in Altiplano and Quiche
Pastoral de la Tierra, Catholic Church NGO	Community development
Proyecto Integrado de Desarrollo Ecoturismo de El Estor-Izabal, NGO supported by grants from Programa de Pequeña y Mediana Empresa (IFC/FMAM)	Broad set of projects
Asociación de Desarrollo Integral Sostenible, ADISE	Tilapia and other enterprises
UNIPESA, Aquaculture and Fisheries unit of the Ministry of Agriculture of Guatemala	Fish culture training and some extension
Viejo Quetzal, NGO supported by the Catholic church	Assist campesinos displaced by internal war
Vivamos Mejor, Swiss NGO	Advise fishermen in Atitlán Lake, tilapia culture in cages

Table 1 summarizes some of the many nongovernmental organizations (NGOs) that work in Guatemala supporting fish culture in the study locales. It is notable that some of the NGOs are church affiliated, some are indigenous to Guatemala, some have ties to large international organizations, and yet others have

focal sources of support in a single developed country. The private nonprofit sector is often one of the most dynamic forces for development in rural Guatemala. Yet, as the case studies will reveal, their technical support in aquaculture is often uneven in quality, selectively available, and sporadic in delivery.

METHODS

Case Communities

La Bendición (The Blessing) is a community in the Pochuta municipality, Chimaltenango department in Central Guatemala. Similarly, San Francisco Ixquiac community is in the Coloma Costa Cuca municipality in Quetzaltenango department. La Bendición and San Francisco Ixquiac were two purposively selected, representative Guatemala communities identified for the study. La Bendición and San Francisco Ixquiac are the specific indigenous communities that have practiced fish culture for over two years.

The worst indices of extreme poverty and absence of social services (human development indicators) are found in the departments of Totonicapán, Huehuetenango, Quetzaltenango and San Carlos where 70% of the population are indigenous (Stavenhagen, 2002). About 65% of the population in Chimaltenango is indigenous.

Based on published statistics, information gained from extensionists and other informants, La Bendición and San Francisco were identified as communities populated largely by indigenous people where fish culture had been practiced for at least two years.

We conducted group and individual interviews in each community. The guiding questions for the study centered on understanding the motivating interests, barriers, and appropriate intervention points for aquacultural development in indigenous communities.

The design of this study follows the data triangulation research strategy described by Yin (1989). Producers and their household members, NGO extension personnel, government technicians, and host-country personnel were interviewed. One of the major constraints is that producers do not keep any records on their production. Participant interviews were guided a general set of questions used to cover the same topics with all participants. The interview guide outlined focal questions about the prospects for fish culture in a locale, particularly in the context of previous experiences or knowledge.

ANALYSIS

The context for tilapia culture in each community is described in some detail, focusing on the problems and circumstances reported by growers and other informants in each locale. Of particular interest are the verbatim expressions of the participants about fish culture and the ways it might be realized in indigenous communities. Fig. 1 shows the location of the study department.

RESULTS

La Bendición

In La Bendición, tilapia cultivation is conducted on communal lands of 10,000 m<sup>2</sup>. Those leading of the project go to the ponds every day; they take turns in groups of two or three people. The ponds are about 500 m from the community by way of a dirt road that is accessible during the whole year.

A fence with a single entrance, a mesh door with padlock, defines the pond site. Although the fence discourages predators and theft, the main intent is to keep children from entering



Figure 1. Map of Guatemala showing study area departments

to play since they would be in danger if they fell into a pond. Three ponds, two are 4 x 4 m and one is 15 x 8 m, are supplied with water from a spring. The water arrives by gravity through polyducts (flexibility black plastic pipes), which reduces costs since is not necessary to pump water. The group purchases fingerlings from the experimental station at Amatitlán, managed by the Universidad de San Carlos de Guatemala, where sex-reversed and mixed-sex fingerlings are sold.

During the cultivation cycle they have gained experience with feeding (frequency, portion), quality of the water, growth of the fish, and other aspects of fish culture. Based on this experience, they now feed three times a day (7:00, 12:00, 17:00) until satiation. This could become a problem, as over or under feeding may elevate production costs.

The high price of concentrate is a problem. Used mainly for the beginning stages, feed with a high percentage of protein (38%) is necessary for fingerlings to become juveniles. The cost is greater than the price of feed used for the next stage of growth (32%) and final grow out (28%). According to the project leader, they have only bought the latter two types of concentrate, as the highest protein feed is too expensive.

The water supply to the ponds is a good quality natural source. It does not flow by other farms or communities, so it is not contaminated with agrochemicals or garbage. Producers hardly ever add replacement water. Additional water flows into the pond, but is not a large amount. The quality of the water during the first months (2–3 months) of cultivation has not been a problem. In the final stage of the cycle, certain incidents have led to excessive primary production (meaning low of oxygen). This problem is likely due to poor handling of the feed, as well as of the high stocking densities. Since the quantity of oxygen required by growing fish increases, they also excrete more

waste. The subsequent algae bloom competes for oxygen at night and it is for that reason that at the end of the cultivation the water of the ponds has a color of green moss.

The cultivation cycle take six months to obtain a 450 g fish, but most producers harvest a 225 g fish. They have not continued fattening the fish as most consumers in the community prefer to buy a kilogram has four or six fish, so that each family member receives and individual fish.

### Fingerlings

The La Bendición group buys fingerlings from Amatitlán; a station managed by the Universidad de San Carlos de Guatemala, specifically CEMA. It takes an hour to transport the fingerlings of the station from Amatitlán, in sealed bags with oxygen. At this station, sex-reversed fingerlings are sold (males), as well as mixed-sex fingerlings (males and females). The group in charge of the ponds bought mixed-sex fingerlings. Consequently, they began to observe reproduction in the pond and diverging variation in the size of the fish.

The group buys fingerlings of *Tilapia Oreochromis niloticus*, the kind they perceive as the easiest to culture, best adapted to their situation, resistant to illnesses, and having the quickest growth. In the beginning, the fingerlings are approximately three grams. One problem is that the fingerlings are mixed-sex. Subsequent reproduction in the pond causes overcrowding, stunting, and variation in the size. The residents think that this it is an opportunity to obtain their own seed and to avoid the expense of the seed purchase. Those advising the group believe it very important, that they buy only sex-reversed fingerlings, to ensure larger, even-sized fish crops. Nonetheless, producers remain attracted to mixed-sex fingerlings by the price difference. Perhaps, they also have doubts about the actual uniformity of the male fingerlings they might purchase.

The producers report stocking 15 fingerlings/m<sup>2</sup>, a relatively high density for the size of the ponds and the level of management possible for the water. Aeration is not possible and replenishing the ponds is difficult. Consequently, the fish have oxygen problems, as farmers report frequent surface piping by the tilapia seeking oxygen. Also due to the lack of water exchange, producers report a high concentration of algae. Asked about water exchange, they said, "We never do replacement of water."

Another problem is that the group has little equipment to manage the ponds. They only report having a scale to weigh the fish for sale, a pail to transport the fish, and a harvest net under poor repair. The group has no means to test water quality, pump water, conduct aeration, or a transport to take product to larger markets.

### Extension

The last time, and one of the few times, that they had contact with a government's extent agent was more than six months ago. They mention that the government extension agent only visited at the beginning of the project, never returning to follow up on their progress. The La Bendición producers say that they never have had contact with an extension representative of the university, neither with any Peace Corps volunteers; they only had the help of the organization "KABAWIL" (NGO). This organization provides the materials to begin the aquaculture project and advises on the management of the pond. The

residents would like to have a more frequent contact with a technical advisor for aquaculture. Regular visits can help them manage their ponds, as well as to undertake improved methods of fish cultivation.

### Marketing

The three ponds have never been totally harvested; only partial harvesting is done to fulfill the needs of a buyer or member of the community. One of the reasons is that they lack the larger net that would be necessary to harvest the whole pond.

The fish are sold at a weight of about 225 grams. Most people prefer fish at this weight, because they obtain more individual fish per kilogram. At meal times, family members then receive individual fish. All the sales are cash; credit is never given. The typical selling price is GTQ 22 per kilogram (GTQ 7.55=US\$ 1.00). Previously they were selling the tilapia for GTQ 17.6 per kilogram, but at this price most producers could not cover costs.

Most of time, fish are sold to neighbors or to others from nearby communities. Middlemen or merchants never buy fish, as the community lacks a good road. The present road is in a bad state, and on some occasions is impassable. In addition to poor road access to the community, it has not been possible to sell the product to any restaurants. Because of the small quantities that have been obtained, the fish are primarily sold inside the community. Fingerlings have not been sold to other communities. The fingerlings that are obtained from reproduction in the ponds are used for production in other ponds.

The La Bendición group harvests and sells the tilapia themselves. They do not hire labor to carry out this task. They do not hire others, in part, because the partial harvests are small. They can only get 4.55 kg to 9.09 kg because they lack labor to conduct larger harvests and they do not have a market large enough to absorb larger quantities of fish.

### San Francisco Ixquiac

The community is located to 55 km from the department seat of Quetzaltenango, one kilometer off the highway that connects Colomba to Coatepeque. In contrast to La Bendición, the community has year-round access to the paved road. The community comprises 45 families, each with an average of five children. At the moment, the village is very organized and has good leadership. Most of residents of the community are devoted to wage labor on lands held by others. They receive the minimum daily wage (currently set at about GTQ 38.75) and work 10–12 hours every day. The houses are constructed with building block and sheet metal roofs. Although the homes tend to have earthen floors, they have electricity and plumbing.

The members of the community cultivate medicinal plants for sale to a vendor who processes and markets the materials collected by the residents. This is carried out as a communal activity with the purpose of obtaining funds to be able to invest or to make arrangements in the community. Some households raise animals such as chickens and pigs, but on a small scale. For example, each household might have 10 chickens and 3 or 4 pigs.

The community has raised tilapia for about two years in a concrete tank, stocking it at a rate of 25 fingerlings/m<sup>2</sup>, a high density for the size of the pond. The pond was built with the collaboration of all the members of the community, each

household contributing construction materials like sand, cement, block, lime, tubes, tools, as well as providing labor. They followed the example of other communities in designing the pond. Making their own plan, they constructed their first pond and obtained satisfying results. The community decision to undertake fish culture was a very important process. Beyond the fish it may provide, the fish pond seems to have symbolic value as a step toward the progressive social and economic development of the community.

### Pond Management

The area of the pond is 60 m<sup>2</sup>, supplied by a spring about 60 m away that belongs to the community. The water arrives by gravity and is of good quality. The source has physical-chemical parameters inside the ideal ranges to undertake the cultivation of tilapia. On one visit, pond water temperature was 25.5°C, oxygen was 2.80 mg/l, and the pH was 6.5. Thus, conditions were generally favorable for tilapia.

The water for the pond comes from a nearby spring that is also used to fill the tank that the women of the community use to wash clothes. The pond is not aerated. Water inflow refills the pond about every 15 days. Consequently, primary productivity is often a source of oxygen depletion for the fish.

The equipment for fish culture possessed by the community San Francisco Ixquiác is minimal. They have only a capture net to harvest fish at the moment of selling them and a scale to weigh them. They do not have vehicles for fish transport, no pushcart, no air pump, and no oxygen meter is available. Members of the community take turns supervising the pond. Each watch obligates the member to visit the pond three times a day every day, staying about 30 to 60 minutes in each visit. They visit the pond to feed the fish three times a day (7:00 AM, 12:00 PM and 5:00 PM). They observe the behavior of the fish, check that they are feeding, and note the color of the water (they never fertilize). These management steps are facilitated because the pond is located amid the community. Such a location also helps avoid theft of the fish. The area is fenced to avoid accidents, as some curious child could fall in the pond. With the use of purchased fish feed, they have problems of high prices, mainly for the beginning stages because it has a high percentage of protein (38%). This feed is more costly than that used for the juvenile stage of growth (32%) and final grow out (28%). They use a feed formulated for tilapia, although they buy the cheapest (GTQ 5 per kg) 32% protein. For the final stages of the cultivation cycle, they buy a concentrate that has 28% protein (GTQ 4.4 per kg).

Weekly, they feed 47.2 kilograms of feed over a cultivation cycle that lasts seven months. They obtain fish with an average weight of 400 grams. In previous production cycles, they have used around 1,336 kilograms of concentrate until the previous day of the harvest, obtaining overall yields up to 359.1 kilograms of fish. Because they do partial harvest, they do not need to hire labor. Two people can accomplish restocking and other operations, therefore they do not need to hire labor to harvest or otherwise maintain the pond.

With the concrete tank, they have obtained good results. Subsequently, they built a new pond on a thousand square meter plot of communal land to be filled with water from a nearby well. Problems with the pumping equipment prevented its use. The pond was 15 x 20 m, covered with nylon netting for

bird protection. The plan was to fill the pond with water from a well a few meters away with a pump. The company that sold the equipment told them that everything would work well, but when it came time to install the pump and put it to work, it was discovered that the motor did not have enough power to pump the water.

The company did not return the money, nor did it exchange the equipment. At the end the community lost their investment. Furthermore, the reservoir was not well built and was too deep. When ask about the problems, one community leader said, "We made it this way because we did not know how to build it, nor did we know what depth to make it, and we thought, the deeper the better." After this bad experience, they nonetheless plan to buy land near the concrete tank to build more ponds there. This would enable them to employ the water source that already supplies the existing, working pond. They do not have problems with polluted water at this time because the source is a natural well, but they are knowledgeable about the importance of protecting their water resource.

### Fingerlings

The residents of the community San Francisco Ixquiác acquire tilapia fingerlings from a private farm "Santa Rita". It sells sex-reversed and mixed-sex tilapia fingerlings. Its brood stock is based on YY-technology super males (Mair et al., 1997). The fingerlings are sex-reversed and have a price of GTQ 0.60 per fingerling. The fingerlings in this farm are more expensive than those of the station of Amatitlán/CEMA. They said that they decided to use this type of fingerling because of its resistance to diseases, hardiness, and rapid growth.

### Extension

They have received technical advice from the municipality of Colomba Costa Cuca, one of the best-organized municipalities in the department of Quetzaltenango. Nonetheless, the municipality does not have an extension agent focused on aquaculture. The municipality depends on a project superintendent to make visits to the communities to help solve problems. This person has minimal knowledge in the field of aquaculture. The municipal technician visits the community about every two weeks.

Having little contact with government, aquaculture stations or extension agencies, the residents know that good pond management is important. Nonetheless, they have made mistakes in pond construction and other areas where they did not have guidance at the right time. The only time that they have had contact with technical staff of the university was during the fieldwork for the case study. Technicians from the Universidad de San Carlos de Guatemala and the Centro de Estudios del Mar y Acuicultura visited the community to conduct interviews for this study. They also visited the ponds and made some recommendations on the cultivation of tilapia.

The residents reported that they need more information about pond management. In particular they wanted to know more about fish nutrition, water quality, reproduction, characteristics of tilapia, pond construction, and anything related to the cultivation of tilapia. Tilapia projects like this are very important (in the words of the village leader), because they aid in the feeding of each family, giving them a quality food because of its high content of protein, as well as the possibility for cash sales at the end of each growout cycle. The community also had some fin-

gerling mortality problems due to bad handling. They have not been selling fingerlings to other fish farmers because producers prefer monosex batches of uniform sizes to stock their ponds.

### Marketing

The pond group has never had a complete harvest of a fish crop; instead they conduct partial harvests when customers arrive to buy the fish. Fish are marketed at an average weight of 225 grams, approximately four to five fish per kilogram. Transactions are cash and no credit is extended under any circumstances. The fish are weighed onsite and a price of GTQ 22 per kilogram charged to the members of the community. To outsiders, GTQ 26.4 per kilogram is the price of tilapia. The community follows this practice so that the members of the community can have the possibility of acquiring protein at a lower price. They do not know if any of their buyers are middlemen, because they do not ask if the buyer is a final consumer or a middleman.

Sales to restaurants have yet to take place, because they have made sales to community members and to outsiders, and little is available for sale in larger quantities. Marketing of the small quantity produced so far has not been a problem. Lack of transportation does inhibit their ability to seek different markets and sell to restaurants. They do not have equipment to transport, preserve a fresh appearance, and make a good presentation to the consumer. Consequently, pond bank sales for cash are the main marketing method for this community's fish crop.

### CONCLUSION

The guiding questions for the study centered on understanding the motivating interests, barriers, and appropriate intervention points for aquacultural development in indigenous communities. The communities that practice fish culture seem to take pride of communal accomplishment obtained by developing the pond site and rearing repeated crops of fish. The economic returns were not estimated by this study, they are unlikely to be competitive with the cost of the capital employed. The amount of fish produced, even if sold at prevailing prices would not produce a return sufficiently motivating to most commercial farmers. Nonetheless, the enthusiasm and pride that residents expressed about their fish ponds reflects the community development achievements represented by the pond.

The community achievement embodied in a functioning pond that periodically yields fish was sufficiently motivating for the La Bendición community to build an additional pond. Despite the ponds poor construction, lack of gravity flow water, and the disappointment stemming from the ill-functioning pump, participants seemed eager to find another way to build additional ponds in a more appropriate location.

While lauding the enthusiasm exhibited in these communities, NGOs and other advising indigenous rural communities in Guatemala should pay close attention to site selection and the community context for tilapia culture. Pumping costs are not typically part of an economically sustainable approach to tilapia culture in cool water areas. Water supplies should be sufficiently malleable and reliable to enable producers to fill and replenish ponds whenever the water is needed. Thus, local springs, streams, and irrigation schemes represent possible sources of water for pond aquaculture in indigenous communities. We expect that many indigenous communities could benefit

by having better quality technical services and information to guide decisions for the adoption of tilapia culture and actual implementation of the enterprise. The case studies reveal the limited knowledge that producers have about the biological and chemical processes that are central to pond management and fish culture. To ensure that communities are getting sound advice, NGO technicians should have certified training in pond aquaculture. At the very least, village level workers should have access to a qualified person on the organization staff to conduct internal training, advise on specific projects, and provide overall guidance for aquaculture promotion.

The ACRSP program in Central America has trained over 500 individuals in the selection and management of tilapia broodstock, in techniques for tilapia reproduction and fingerling production over the past five years. It has been clear that trained individuals and competently advised fish producers also need institutional support to ensure that the aquaculture industry develops on a sound footing. The subsets of indigenous communities that own the land and water resources, and water temperatures appropriate to pond aquaculture also need access to fingerlings of good quality for growout. Ensuring that all-male fingerlings live up to their claims, that handling procedures ensure survival on delivery to farm ponds, and that better adapted strains are available to producers is a task for the public sector to foster in the private market.

Universities and government agencies should focus on efforts to improve the national pool of genetic materials and provide technical support to the network of fingerling suppliers that is slowly developing across Guatemala. One important function relates to information, that helps growers know who has the quantity and the kind of fingerlings needed, and helping hatchery managers know how to properly handle and maintain their broodstock and fingerlings.

These activities will enhance institutional understanding of the status of small-scale tilapia culture in Guatemala and the region by building the market for fingerlings and increasing the confidence and effectiveness of growers in indigenous communities. It is only by repeated successful experiences in growing, selling, and consuming tilapia that a market will be built and an industry developed. By this means, income will increase, food security will be increased, and the rural sector advanced.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## FIRST ANNUAL SUSTAINABLE AQUACULTURE TECHNOLOGY TRANSFER WORKSHOP

*Twelfth Work Plan, Sustainable Development and Food Security 4 (12SDF4)  
Abstract*

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### **ABSTRACT**

A series of workshops on indoor, water-recirculating aquaculture systems were conducted. A one-day workshop was held at Hermosillo on 28 November 2005 (approximately 200 people were in attendance); a two-day workshop in March 2006 (approximately 20 people in attendance); and a four-day workshop held in July 2006 at the Universidad Autónoma Metropolitana Unidad Xochimilco (Mexico City) (approximately 20 people in attendance). A three-day September workshop has been planned (80 people pre-registered) to be held in Veracruz in conjunction with the ISTA 7 (international tilapia conference) and Instituto Tecnológico del Mar. Each of these workshops was very successful with high ratings on their quality. Each had a major focus on commercial production systems, both large-and small-scale.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## 5<sup>TH</sup> INTERNATIONAL AQUACULTURE EXTENSION COURSE IN THE AMAZON REGION AND 1<sup>ST</sup> MEETING FOR THE AMAZON REGION AQUACULTURISTS

*Sustainable Development and Food Security 5 (12SDF5)  
Final Report*

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### **ABSTRACT**

The Fifth International Aquaculture Extension Course in the Amazon Region and First Meeting for the Amazon Region Aquaculturists are part of a series of events since 2002 that have been successfully organized in the Amazon region by Southern Illinois University Carbondale (SIUC). The outreach activities have been implemented with the collaboration of several Amazon institutions and funded partially by the United States Agency for International Development (USAID) and the Aquaculture Collaborative Research Support Program (ACRSP). Two intensive training courses (one for producers and indigenous communities and another for professionals and students) were presented to a large group (124 participants, 20 females and 104 males: 65 indigenous community and small-scale producers, 59 students and professionals) of governmental and non-governmental personnel conducting aquaculture research and/or extension activities in Bolivia, Brazil, Colombia, Ecuador, Peru, or Surinam. Both events were held from 11–15 April 2006 in the Macas Saecian Institute and the Voz del Upano conference auditorium in the City of Macas, Ecuador. For each course, 10 qualified candidates from Ecuador's neighboring Amazon countries were invited to participate, as well as a similar number from Ecuador. The main objectives of these courses were to: 1) train participants on the use of technological tools (pond construction, broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, and disease prevention and treatment); and 2) facilitate the exchange of strategies,



experiences, and learned lessons on rural aquaculture extension for the management and reproduction of native Amazon species (i.e., *Colossoma* sp., *Piaractus* sp., *Arapaima gigas*, *Prochilodus* sp., *Brycon* sp., and *Ampularia* sp.). A CD-ROM displaying all the course material for the Amazon aquaculture-training course was also produced.

### INTRODUCTION

Fish culture has been practiced for over three decades in the Peruvian Amazon and for over 50 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. Southern Illinois University Carbondale (USA) was the international coordinating institution for the 5<sup>th</sup> International Aquaculture Extension Course in the Amazon Region and the 1<sup>st</sup> Meeting for the Amazon Region Aquaculturists; the NGO Fundación Ecológica Arcoiris, under the Sustainability Program and Regional Union (PSUR in Spanish), in Morona Santiago (Ecuador), was the national coordinating institution. The PSUR initiative is part of the bi-national peace plan between Ecuador and Peru, funded by USAID. During the past 5 years, Arcoiris has facilitated research and development in the aquaculture sector in the southern Ecuadorian Amazon region.

The following events are part of a series that have been successfully organized in the Amazon region by a group of institutions since 2002:

- I Curso internacional de acuicultura para productores y extensionistas en la Amazonía, held in Iquitos (Perú), from 25–30 April 2002. Sponsors/Organizers: CRSP (USAID), IIAP, UNAP y Gobierno Regional. Participating countries: Brazil, Colombia, Ecuador and Peru (19 participants).
- II Curso internacional de acuicultura para extensionistas de la Amazonía, held in Iquitos (Peru), from 25–30 August, 2002. Sponsors/Organizers: CRSP (USAID), IIAP, UNAP y Gobierno Regional. Participating countries: Bolivia, Brazil, Colombia, Ecuador and Peru (23 participants).
- III Curso internacional de acuicultura para extensionistas de la Amazonía, held in Pucallpa (Perú), from 18–21 August 2003. Sponsors/Organizers: CRSP (USAID), IIAP, UNAP, Gobierno Regional, Marina de Guerra Participating countries: Bolivia, Brazil, Colombia, Ecuador, Peru and Venezuela (63 participants).
- I Curso Internacional de Nutrición de Peces Tropicales, held in Pucallpa (Perú), on 22 August 2003. Sponsors/Organizers: CRSP (USAID), IIAP, UNAP, Gobierno Regional, University of Arkansas. Participating countries: Bolivia, Brazil, Colombia, Ecuador, Peru and Venezuela (63 participants).
- IV Curso internacional de acuicultura con especies promisorias de la Amazonía – Professionals and students. Leticia (Colombia) / Benjamín Constant (Brazil), from 21–24 July 2004. Sponsors/Organizers: CRSP (USAID), UNAL, Alcaldía and Gobernación, Sinchi, Acuorios Leticia, INPA, Incoder, Acuiamazonas, IDAM. Participating countries: Brazil, Colombia, Ecuador, Peru and Venezuela (57 participants).
- IV Curso internacional de acuicultura con especies promisorias de la Amazonía –Productores, held in Leticia (Colombia), from 22– 24 July 2004. Sponsors / Organizers: CRSP (USAID), UNAL, Alcaldía y Gobernación, Sinchi, Acuorios Leticia, INPA, Incoder, Acuiamazonas, IDAM. Participating countries: Brazil and Colombia (20 participants).

- I Curso internacional de Cultivo de Peces Ornamentales, held in Leticia (Colombia), from 25–27 July 2004. Sponsors/Organizers: CRSP (USAID), UNAL, Alcaldía and Gobernación, Sinchi, Acuorios Leticia, INPA, Incoder, IDAM and Acuiamazonas. Participating countries: Brasil and Colombia (18 participants).

The addition of South America to the PD/A CRSP in the eight work plan has provided considerable and unique opportunities to expand the CRSP Central Database. In the ninth work plan, a prime site was established at Iquitos, Peru, which is in the heart of the Peruvian Amazon (Loreto Region).

In eleventh work plan, new sites (Brazil, Colombia, and Ecuador) were added to expand the project to be more inclusive of the Amazon Basin. The major Brazilian Amazon institutions currently conducting aquaculture outreach and research are the Instituto Nacional de Pesquisas da Amazonia (INPA) and Universidad Federal do Amazonas (UFAM). In the Ecuadorian Amazon, the NGO Arcoiris is the leading aquaculture extension institution in the region.

In the Colombian Amazon, the institutions conducting research in aquaculture are: Universidad Nacional (UNAL), Instituto de Investigaciones de la Amazonia (SINCHI) and Corporación Regional del Amazonas (Corpoamazonia). Considerable potential exists to examine other species, as the Amazon Basin is home to over 2,000 freshwater species of fish and innumerable invertebrates.

A Memorandum of Understanding (MOU) is currently in place linking IIAP, INPA, UFAM, UNAL, Arcoiris, Peace Corps Ecuador, and SIUC (and collaborating US universities) into the CRSP network. Facilities have been significantly upgraded/renovated at IIAP during the ninth, tenth, and eleventh work plans. Outreach and networking activities have been greatly expanded during the twelfth work plan to facilitate regionalizing the benefits of the CRSP to nearly every country comprising Amazonia.

### MATERIALS AND METHODS

**Objective 1: Train participants on the use of technological tools; facilitate the exchange of strategies, experiences, learned lessons on rural aquaculture extension for the management and reproduction of native Amazon species.**

Two intensive training courses (one for producers and indigenous communities and another for professionals and students) for a large group of governmental and non-governmental personnel conducting aquaculture research and/or extension activities in the Amazon Basin were offered at the Macas Salecian Institute and the Voz del Upano conference auditorium in Macas, Ecuador, from 11–15 April, 2006. This training plan was a continuation of the very successful program that has trained several extensionists from Bolivia, Brazil, Colombia, Ecuador, and Peru. For each course, 10 qualified students, along with several NGO personnel and indigenous community

representatives from Ecuador's neighboring Amazon countries, were invited to participate, as well as a similar number of representatives from Ecuador. The course was offered to train aquaculturists and experts in aquaculture related degrees in extension techniques that have been practiced successfully by IAP and Terra Nuova, including CRSP's new experiences in the region through the eleventh work plan. Extension personnel learned extension techniques, biosecurity in aquaculture, pond construction, broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, and disease prevention and treatment, all specifically related to the native cultured species of *Colossoma sp.*, *Piaractus sp.*, *Arapaima sp.*, *Prochilodus sp.* and *Brycon sp.* (fish), and mollusks (Congompe and Churo). During a one-day practical training session, the participants learned hormone injection, spawning, fertilization, incubation and larviculture techniques. A CD-ROM displaying all the course material for the Amazon aquaculture-training course was also produced.

## RESULTS

**Objective 1: Train participants on the use of technological tools, and facilitate the exchange of strategies, experiences, learned lessons on rural aquaculture extension for the management and reproduction of native Amazon species.**

The two intensive training courses were presented to 124 participants (20 females and 104 males of which 65 represented indigenous community and small-scale producers, and the remainder, 59, students and professionals), all of who conduct aquaculture research and/or extension activities in Bolivia, Brazil, Colombia, Ecuador, Peru or Surinam. Both events were held from 11–15 April 2006 in the Salecian Institute and the Voz del Upano conference auditorium in the City of Macas, Ecuador. The main objectives of these courses were to: 1) train participants on the use of technological tools (pond construction, broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, disease prevention and treatment); and 2) facilitate the exchange of strategies, experiences, and learned lessons on rural aquaculture extension for the management and reproduction of native Amazon species (i.e., *Colossoma sp.*, *Piaractus sp.*, *Arapaima gigas*, *Prochilodus sp.*, *Brycon sp.*, and *Ampularia sp.*). A CD-ROM displaying all the course material for the Amazon aquaculture-training course was also produced.

## DISCUSSION

The International training courses gave continuity to the channels initially opened by CRSP in the tenth and eleventh work plans for the creation and support of a network of aquaculturists in the Amazon Basin. Some of these aquaculturists gained expertise to function more efficiently in extension or production activities.

## 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 benefited 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 over harvested, 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 Gouling, 1997). 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 benefited 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 quantified the following:

- 124 participants (20 females and 104 males: 65 indigenous community and small-scale producers, 59 students and professionals) of governmental and non-governmental personnel were trained.
- Personnel from six Amazon Basin countries participated in the training courses.

## CONCLUSIONS

The International training courses gave continuity to the channels initially opened by CRSP in the tenth and eleventh work plans for the creation and support of a network of aquaculturists in the Amazon Basin.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## NEW PARADIGM IN FARMING OF FRESHWATER PRAWN (*MACROBRACHIUM ROSENBERGII*) WITH CLOSED AND RECYCLE SYSTEMS: THAILAND

*Twelfth Work Plan, Production System Design and Integration Research 1a (12PSD1a)*  
Abstract

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### ABSTRACT

This study included two parts: an experiment on a water recycling system for giant freshwater prawn (*Macrobrachium rosenbergii*) and a survey of prawn farming systems in Thailand.

The experiment was conducted in 15 cement tanks (2 x 2.5 x 1 m) at the Asian Institute of Technology, Thailand, during 5 January to 12 May 2004, to develop closed and recycle systems for culture of giant freshwater prawn. Juvenile prawns were cultured in three systems as three treatments, each in triplicate: (A) open system with water exchange, (B) closed system with aeration, and (C) recycle system, in which water from a prawn tank was circulated through a Nile tilapia (*Oreochromis niloticus*) tank to a water mimosa (*Neptunia oleracea*) tank and back to the prawn tank.

Survival of prawns, ranging from 40.6% to 88.7%, was highest in the closed system, intermediate in the recycle system, and lowest in the open system ( $P < 0.05$ ). Growth of prawns was not significantly different among all three systems ( $P > 0.05$ ), while gross and net yields of prawn were significantly lower in the open system than in closed and recycle systems ( $P < 0.05$ ). Feed conversion ratio (FCR) in the open system was 2.81, which was significantly higher than in the closed (1.67) and recycle (1.78) systems ( $P < 0.05$ ). Prawn recovered 12.02% N and 7.01% P from feed and fertilizer in the open system and 25.26% N and 13.67% P in the closed system. Prawn, tilapia, and water mimosa together recovered 39.55% N and 25.53% P in the recycle system. Economic analyses showed that there were no significant differences in net returns among the three systems.

The socioeconomic and technical survey of 100 prawn farmers was conducted during 1 May–31 July 2005 in Thailand. Survey results showed that the majority of respondents were male (70%) and ranged in age from 19–72 years, average age 46. Most farmers (77%) had completed an elementary level of schooling (4 years), 16% had completed high school (12 years), 6% had vocational/university education, and 1% had no formal education. Experience or length of time working on the farm as owner, manager, or both ranged from 8 to 25 years, averaging approximately 10 years. Formal training was received by 19% of respondents and most (92.9%) obtained information about prawn culture from their neighbors.

The majority of farms were under 5 ha in both total and water area. Monoculture was the dominant system (96%) while remaining farmers utilized polyculture systems consisting of prawns and white shrimp (*Metapenaeus vannamei*). The most common management strategy (66%) included nursing post larvae (PL) and harvesting with the combined method where farmers cull only marketable sized prawns after five months and allow those stunted by dominants to grow and be harvested on a 30–45 day basis. After several harvests, ponds are drained and all prawns are harvested. Other strategies included stocking PL or juveniles directly into grow-out ponds and using the batch method, where all individuals are harvested after reaching a medium market size.

Semi-intensive culture was predominant. Most farmers stocked at densities below 20 pcs m<sup>-2</sup>, average  $11 \pm 1$  pcs m<sup>-2</sup> (72%,  $n = 75$ ). Transfer survival values (from the nursing pond) were transformed to the natural log to correct for normality and this variable was significantly correlated to stocking density (slope = 0.012,  $P < 0.05$ ,  $R^2 = 0.599$ ). Average production was  $2155 \pm 146$  kg ha<sup>-1</sup> yr<sup>-1</sup> and ranged from 438 to 6381 kg ha<sup>-1</sup> yr<sup>-1</sup> ( $n = 72$ ). Major problems identified were diseased or poor quality seed supply (67%), disease outbreak within the crop (64%), and external pollution (37%). External pollution was severe for 16% of respondents, moderate for 46%, and not an issue for 38%. A linear regression model is being developed to establish which variables are most important in driving production and net economic profits.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## NEW PARADIGM IN FARMING OF FRESHWATER PRAWN (*MACROBRACHIUM ROSENBERGII*) WITH CLOSED AND RECYCLE SYSTEMS: VIETNAM

*Twelfth Work Plan, Production System Design and Integration Research 1b (12PSD1b)  
Abstract*

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### ABSTRACT

Two surveys on giant freshwater prawn (*Macrobrachium rosenbergii*) farming were conducted in the Mekong Delta, Vietnam. The first survey was carried out during March–April 2005 and the second during May–June 2006. These two surveys were conducted in the same locations. Forty-seven prawn farmers were randomly selected during the first survey, among whom 15 farmers were from Co Do district of Can Tho city, 15 farmers from Vinh Thanh district of Can Tho city, and 17 farmers from Thoai Son district of An Giang province. For the second survey, 20 farmers were selected from Co Do district, 16 from Vinh Thanh district, and 20 from Thoai Son district. The selected farmers were interviewed using a structured checklist and open-ended type of questionnaire. The surveys focused on prawn farming in rice paddies to assess the changes of giant freshwater prawn farming including development trends and technical, socio-economic, and environmental aspects.

The results showed that prawn farming in rice paddies in the Mekong delta is continuing to expand. There were improvements of culture techniques between two surveys in terms of rice paddy design and construction, stocking density, feeds and feeding, water quality management, productivity, profitability, and so on. Detailed data analyses are ongoing and detailed results will be included in the final report.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## NEW PARADIGM IN FARMING OF FRESHWATER PRAWN (*MACROBRACHIUM ROSENBERGII*) WITH CLOSED AND RECYCLE SYSTEMS: BANGLADESH

*Twelfth Work Plan, Production System Design and Integration Research 1c (12PSD1c)*  
Abstract

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### ABSTRACT

The study was conducted to understand the status and practices of freshwater prawn production systems in four different zones of Bangladesh during January to July 2005. A total of 100 farmers were interviewed with a semi-structured questionnaire and participatory rural appraisal tools on prawn post-larvae (PL) nursing and grow-out rearing in ponds or ghers.<sup>1</sup> In nursing systems, the farmers reared post larvae in small ponds or in the trench of ghers with water area ranging from 5 to 30 decimals (average 8.29 decimals). About 49% of farmers stocked hatchery PLs due to shortage of supply and high price of wild PLs. Almost all farmers dried and limed their nursery ponds or pocket ghers, repaired their dikes, and removed aquatic weeds in the dry season. Farmers used urea, TSP, and cow dung. The stocking density of PLs was 400 to 1500 individuals decimal<sup>-1</sup>. The mean quantity of supplied feeds was 36.97 g per 1000 PLs day<sup>-1</sup> for the first 15 days, 72.11 g per 1000 PLs day<sup>-1</sup> for the second 15 days, 125.59 g per 1000 PLs day<sup>-1</sup> for the third 15 days, and 205.15 g per 1000 PLs day<sup>-1</sup> up to the juvenile stage. The PL mean survival rate was 67.5%.

In the grow-out farming system, farmers reared juvenile prawns either in ponds and/or ghers. Farm size and individual pond/gher size ranged from 0.08 to 31.5 ha and 0.08 to 3.94 ha, respectively. The gher/pond design provides good opportunities for diversification with primary dependence on prawns, fish, and rice. Thirty percent of farmers did not practice integrated culture, 40% practice integrated prawn with paddy culture, 10% integrated prawn farming with only dike crops, and 20% integrated farming with paddy and dike crops. In all study areas, the peak season of prawn farming was from May to January. Prawn farmers used three different types of supplementary feed (processed feed, homemade feed, and snail meat). Almost all farmers applied feeds at an average rate of 4.5% body weight basis. The peak season of partial harvesting was from October to January and small prawns were reared up to next season and were harvested in the following year from August to September. The average annual yield of prawns, fish, and shrimp was estimated at 390.2 kg ha<sup>-1</sup>, 658.5 kg ha<sup>-1</sup>, and 123.9 kg ha<sup>-1</sup>, respectively. Farmers also cultivated small numbers of silver carp, rohu, catla, and silver barb with prawns mainly used for their own consumption.

Major problems of prawn farming were lack of capital, lack of education, shortage of PLs, high price of quality feed, poor technical knowledge, marketing problems, poor water quality, excessive and late rain, natural disasters (flood and drought), poisoning, water exchange problems, traditional technology, and disease problems. For long-term sustainability of prawn farming in the study area, adequate bank credits at very low interest, sufficient and quality seed production, and improved management skills are needed. Moreover, good transportation, a favorable marketing system, and a positive attitude towards prawn farming should be developed. Training, extension services, and institutional and policy support should be provided to the prawn farmers for sustainable prawn farming in Bangladesh.

<sup>1</sup> The Bangla term "gher" is an enclosure made for prawn cultivation by modifying rice fields by building higher dikes around the field and excavating a canal several feet deep inside the periphery of the dikes to retain water during the dry season.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## OPTIMIZATION OF FERTILIZATION REGIMES IN FERTILIZED NILE TILAPIA (*Oreochromis niloticus*) PONDS WITH SUPPLEMENTAL FEED

*Twelfth Work Plan, Production System Design and Integration 2 (12PSD2)*  
Abstract

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### ABSTRACT

An experiment was conducted in 15 200-m<sup>2</sup> earthen ponds at the Asian Institute of Technology, Thailand from September 2005 to January 2006. The objectives of this experiment were to determine effects of different rates of phosphorus fertilizer application on Nile tilapia (*Oreochromis niloticus*) production, pond water quality parameters, and nutrient utilization efficiency under supplemental feeding and to evaluate the cost and return of Nile tilapia production. Five phosphorus fertilization rates were used as treatments in a randomized complete block design: 100%, 75%, 50%, 25%, and 0% of 7 kg P ha<sup>-1</sup> wk<sup>-1</sup>. Nitrogen fertilization rate was fixed at 28 kg N ha<sup>-1</sup> wk<sup>-1</sup> for all the treatments throughout the experiment. Sex-reversed, all-male Nile tilapia of about 100 g size were stocked at 3 fish m<sup>-2</sup> and fed at 50% satiation feeding rate during the culture period.

Mean weight, mean weight gain, daily weight gain, and net fish yield were not significantly different among treatments ( $P > 0.05$ ). Water quality parameters were not significantly different among treatments, except total Kjeldahl nitrogen, total phosphorus, and soluble reactive phosphorus. Nutrient budget showed that higher rates of phosphorus fertilizer input resulted in higher phosphorus accumulation in the sediments. Economic analysis showed that all treatments with phosphorus fertilization resulted in positive net returns. Gross income was not affected by different phosphorus fertilization rates. Treatment with 25% phosphorus fertilization might be used as an alternative strategy for Nile tilapia pond culture in terms of good economic return and reduced nutrient loss in sediment.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## USE OF RICE STRAW AS A RESOURCE FOR FRESHWATER POND CULTURE

*Twelfth Work Plan, Production System Design and Integration 3 (12PSD3)  
Abstract*

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### ABSTRACT

Rice straw is a low-cost material and widely available in farms from South and Southeast Asia. Rice straw can enhance fish production if used as a substrate in aquaculture ponds because it allows periphyton colonization and also increases benthic organisms on decomposition. The objectives of this study were to investigate the role of rice straw in fish ponds and to develop a low-cost aquaculture system using rice straw to enhance fish production through reduced turbidity, enhanced biofilm, and increased periphyton development. This study was comprised of four experiments that were conducted in Thailand and Bangladesh.

**Experiment 1** was conducted in 21 outdoor cement tanks of 5-m<sup>2</sup> in surface area at the Asian Institute of Technology, Thailand (AIT), for 35 days to assess effects of straw decomposition at various loading levels on physical, chemical, and biological water quality parameters. The treatments were seven loading levels of rice straw mats: 0, 625, 1,250, 2,500, 5,000, 10,000, and 20,000 kg ha<sup>-1</sup> (dry matter basis). Tanks were fertilized weekly with urea and TSP at 28 kg N and 7 kg P ha<sup>-1</sup> week<sup>-1</sup>, respectively. Diel temperature, dissolved oxygen (DO), and pH were monitored every day, while Secchi disk visibility was measured daily at 0900 h. Water column samples were taken weekly at 0900-1000 h for the analyses of total alkalinity, total ammonia nitrogen (TAN), nitrite-N, nitrate-N, total Kjeldahl nitrogen (TKN), total phosphorus (TP), soluble reactive phosphorus (SRP), total suspended solids (TSS), total volatile solids (TVS), chlorophyll *a*, and tannin. Rice straw samples were taken from each tank at the beginning and the end of the experiment to quantify periphyton using Sedgwick-Rafter cells and bacteria number (cfu/g) using total plate counts.

High straw loading rates caused deteriorating water quality. Increased loading rates decreased DO, pH, and transparency ( $P < 0.05$ ), while total alkalinity, nitrite-N, TP, SRP, TKN, TSS, TVS, and chlorophyll *a* increased with increasing loading rates ( $P < 0.05$ ). Periphyton biomass (chlorophyll *a* content, dry matter, ash- and ash-free dry matter), was found to be higher in lower straw-loading treatments ( $P < 0.05$ ). However, plankton (cell/L) and bacteria number (cfu/g) did not differ significantly among treatments ( $P > 0.05$ ). The loading rate of 625 kg ha<sup>-1</sup> was found to be best among treatments.

**Experiment 2** was conducted with different rice straw loading rates in fertilized earthen ponds of 200 m<sup>2</sup> at AIT to assess effects of straw mats on growth performance of Nile tilapia (*Oreochromis niloticus*), pond water quality, periphyton, plankton, bacterial biofilm, and benthos. There were six treatments with three replicates: 1) control (without rice straw mats); 2) rice straw mats of 5 x 0.5 m covering dikes; 3) one rice straw mat of 5 x 1 m in water column; 4) two rice straw mats of 5 x 1 m in water column; 5) three rice straw mats of 5 x 1 m in water column; and 6) four rice straw mats of 5 x 1 m in water column. All ponds were fertilized weekly with urea and TSP at 28 kg N and 7 kg P ha<sup>-1</sup> week<sup>-1</sup>. Sex-reversed, all-male Nile tilapia with a mean weight of 24.7 ± 3.0 g were stocked 39 days after placing rice straw mats in the pond at 2 fish m<sup>-2</sup>.

Growth performance of Nile tilapia was not significantly different ( $P > 0.05$ ) between treatments with straw mats and the control, except the treatment with two straw mats, which had a significantly lower mean weight gain and mean yield than the control ( $P < 0.05$ ). There was no significant difference ( $P > 0.05$ ) in mean survival and yield among the treatments with different straw loading rates. Rice straw loading had no significant affect on the major water quality parameters, plankton density, bacterial biofilm, or benthos. Periphyton samples were collected only during the first month of the experimental period as the rice straw fell off the supporting structure, and thus, it was not possible to collect substrate samples to determine periphyton biomass. A sharp decline in DO was observed in the rice straw treatments after placing rice straw mats in the ponds (pre-stocking period). Eighty-seven genera of phytoplankton were identified with dominant species in the following orders: Bacillariophyceae, Chlorophyceae, Cyanophyceae, and Euglenophyceae. *Cyclotella*, *Microcystis*, and *Euglena* were the dominant genera.

Twenty genera of zooplankton were identified; among those, Rotifera and Crustacea were the most dominant groups, whereas *Brachionus* and *Nauplius* were the dominant genera. Total plate count of bacteria in water did not significantly differ among treatments, but total counts declined towards the end of the experiment. Total benthos count was also not significantly different ( $P>0.05$ ) among treatments, and Oligochaete was the dominant group. Rice straw loading did not enhance growth and yield of Nile tilapia and had no apparent effect on major water quality parameters, plankton community, bacterial growth, or benthos. However, rice straw mat structure collapsed during the early experimental period (15 days after fish stocking), and therefore the full potential of rice straw as a substrate for periphyton attachment could not be evaluated in this study. Further research is required to assess the effect of rice straw loading in fertilized Nile tilapia ponds with a durable rice straw mat structure.

**Experiment 3** was carried out in 18 40-m<sup>2</sup> earthen ponds in a completely randomized design at the Bangladesh Agricultural University (BAU) for 90 days to optimize the loading number of rice straw mats in carp polyculture ponds. The treatments with three replicates are: 1) control (without rice straw mats); 2) rice straw mats covering the slope of dikes; 3) one rice straw mat in the water column; 4) two rice straw mats; 5) three rice straw mats; and 6) four rice straw mats. Ponds were drained, dried, and limed using CaCO<sub>3</sub> at a rate of 250 kg ha<sup>-1</sup>, then rice straw mats (2 x 1 m) were placed into the treatment ponds according to the design. Urea, TSP, and cow dung were applied on the following day at rates of 31 kg ha<sup>-1</sup>, 16 kg ha<sup>-1</sup>, and 1,250 kg ha<sup>-1</sup>, respectively, and continued throughout the experiment on a biweekly basis. After placing rice straw mats, DO was monitored daily at 0600 h. Fingerlings of rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), catla (*Catla catla*), common carp (*Cyprinus carpio*), and silver carp (*Hypophthalmichthys molitrix*) with mean weights of 25.5 ± 0.31, 26.2 ± 0.93, 24.0 ± 0.87, 23.5 ± 0.85 g, and 25.9 ± 0.48, respectively, were stocked at one fish m<sup>-2</sup> one month later after DO recovery at a ration of 3:2:2:2:1. Diel temperature, DO, pH, and Secchi disk visibility were measured weekly, while total alkalinity, TAN, nitrite-N, nitrate-N, TN, SRP, TP, chlorophyll *a*, TSS, and TVS were analyzed biweekly. Periphyton and plankton were quantified following the method in Experiment 1.

Preliminary analyses showed that all measured water quality parameters were found within the desirable range for fish culture. Net fish yield was highest in the treatment with three mats (1.76 tons ha<sup>-1</sup> year<sup>-1</sup>) and lowest in the control (0.9 tons ha<sup>-1</sup> year<sup>-1</sup>;  $P<0.05$ ).

**Experiment 4** is being conducted in nine 40 m<sup>2</sup> ponds at BAU for three months to compare rice straw and kanchi as periphyton substrates to enhance fish production. There were three treatments in triplicate: A) without substrate (control); B) rice straw mats as substrate (three mats per pond, 3 x 625 kg ha<sup>-1</sup>); and C) kanchi as substrate (390 bamboo side shoots per pond). Prior to placing the substrates, ponds were drained and dried, the top layer of sediment was removed and limed using CaCO<sub>3</sub> at a rate of 250 kg ha<sup>-1</sup>. Urea, TSP, and cow dung were applied on the following day at rates of 31 kg ha<sup>-1</sup>, 16 kg ha<sup>-1</sup>, and 1,250 kg ha<sup>-1</sup>, respectively, and continued throughout the experiment on a biweekly basis. Then, 390 kanchi and three straw mats per pond were fixed in the respective treatment ponds. DO in ponds with rice straw mats was monitored for two weeks until it recovered to a normal level. Fingerlings of rohu (24.5 ± 0.5 g), mrigal (25.1 ± 0.6 g), catla (25.8 ± 0.5 g), silver (30.4 ± 0.9 g) and common carp (27.6 ± 0.6 g) were stocked at 1 fish m<sup>-2</sup> with the species ratio of 3:2:2:2:1. Diel temperature, DO and pH were measured weekly, while total alkalinity, TAN, nitrite-N, nitrate-N, TN, SRP, TP, chlorophyll *a*, TSS, and TVS are analyzed biweekly. Qualitative and quantitative analysis of periphyton, plankton, and bacteria is performed monthly. The experiment continues is ongoing.





# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## DEVELOPMENT OF A RECIRCULATING AQUACULTURE SYSTEM MODULE FOR FAMILY/MULTI-FAMILY USE

*Twelfth Work Plan, Production System Design and Integration 4 (12PSD4)*  
*Abstract*

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### **ABSTRACT**

This project is developing a freshwater recirculating aquaculture system for small-scale tilapia production that is appropriate for a family unit. A design and management manual is being developed.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## INSULIN-LIKE GROWTH FACTOR-1 GENE EXPRESSION AS A GROWTH INDICATOR IN NILE TILAPIA

*Twelfth Work Plan, Production System Design and Integration 5 (12PSD5)*  
Abstract

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### ABSTRACT

IGF-I, a mitogenic polypeptide, is an important regulator of growth in fish. The potential of IGF-I mRNA abundance as a rapid growth indicator in Nile tilapia (*Oreochromis niloticus*) was evaluated. Hepatic IGF-I cDNA was isolated and partially cloned. The partial 539-base sequence encodes for the signal peptide, mature protein, and a portion of the E domain. The deduced 68 amino acid sequence for mature IGF-I showed 84–90% and 77–79% sequence identity with fish and mammalian counterparts, respectively. The deduced amino acid sequence for domains B and A was most conserved (93–97%) relative to other fishes. A sensitive TaqMan real time qRT-PCR assay for *O. niloticus* was developed based on the mature IGF-I peptide for measures of hepatic IGF-I mRNA levels. Hepatic IGF-I mRNA levels were found to be significantly correlated with growth rate of fish reared under different feeding regimes and temperature conditions. Higher feed consumption and water temperature produced faster-growing fish and increased hepatic IGF-I mRNA expression. These findings suggest that hepatic-derived IGF-I plays a key role in controlling growth in *O. niloticus* and indicates that IGF-I mRNA quantification could prove useful for the rapid assessment of growth rate in this species.

Additional studies undertaken by our group have shown that IGF-1, and consequently growth, are influenced by temperature, social status, and photoperiod. The growth rate of fish reared in warmer temperatures was significantly increased in a time dependent manner ( $r = 0.93$ ). Mean hepatic IGF-I mRNA levels in fish reared at warm temperature for two, five, and seven days were elevated 1.6-fold, 2.5-fold, and 3.6-fold, respectively, compared to that of fish reared at cold temperature.

A fish's relative position in the social hierarchy consistently influenced levels of IGF-I mRNA in the liver and eye color pattern. Lower social status correlated with depressed hepatic IGF-I levels while dominant status stimulated hepatic IGF-I production.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## DEVELOPMENT OF NILE TILAPIA FILLETS AS AN EXPORT PRODUCT FOR THE PHILIPPINES

*Twelfth Work Plan, Production System Design and Integration 6 (12PSD6)  
Abstract*

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### ABSTRACT

The experiment was undertaken to determine the culture period for Nile tilapia (*Oreochromis niloticus*) to reach an average weight of 600 g at a stocking size of 50–120 g. This is the approximate size required for the production of fillets to serve export markets. The grow-out study was conducted in six 500 m<sup>2</sup> earthen ponds. GET-ExCEL Nile tilapias were stocked at a density of 1 pc m<sup>2</sup> (Treatment I) and 2 pcs m<sup>2</sup> (Treatment II).

Analysis of variance revealed no significant differences in the initial weights of the fish between treatments ( $P>0.05$ ). After a culture period of four months, harvested fish in Treatment I had a mean weight of 590.17 g while in Treatment II, they had a mean weight of 512.99 g. Similarly, there was no significant difference for the mean final weights of fish between treatments. There were also no significant differences in the mean final length, mean survival rates, daily weight gains, specific growth rates, feed conversion ratios, or feed conversion efficiencies of the fish stocks in the two treatments ( $P>0.05$ ). Significant differences ( $P<0.05$ ) were observed between the extrapolated fish yield in Treatment I ( $5,250.93 \pm 313.05$  kg ha<sup>-1</sup>) and Treatment II ( $8,256.43 \pm 423.16$  kg ha<sup>-1</sup>) and on fish biomass in Treatment I ( $219.84 \pm 15.93$  kg) and Treatment II ( $327.77 \pm 21.91$  kg).

The percent fillet recovery was highest in fish sizes ranging from 601–700 g which had a mean value of 36% while fish size ranging from 701–800 g and 501–600 g had 34.99% and 34.03% fillet recovery, respectively. Our economic analysis showed that Treatment I had better cost-benefit ratio compared with Treatment II. This suggests that rearing of Nile tilapia at a density of 1 pc m<sup>-1</sup> was more profitable for the production of tilapia for fillet production.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## TILAPIA-SHRIMP POLYCULTURE IN NEGROS OCCIDENTAL, PHILIPPINES

*Twelfth Work Plan, Production System Design and Integration 7 (12PSD7)*  
*Abstract*

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### ABSTRACT

The tilapia-shrimp polyculture project on Negros Island in the Philippines started quickly, but was then slowed considerably when our industry partner (FYD International) left the project. Students who had begun research were delayed until another farm location was identified with a new industry partner. Our new partner, Cruz Aquaculture, has been a good collaborator and we are back on track and expect to have the research and reporting completed within the period of the extension.

Specifically we will be comparing three different polyculture styles, a sequential method having tilapia in one pond and passing the same water to a shrimp pond, and two simultaneous methods — the first having tilapia in cages in shrimp ponds and the second having tilapia loose in shrimp ponds. We will focus on production results (fish and shrimp yields and survival, feed conversion ratios, and cost and benefits), water quality parameters (dissolved oxygen, secchi disk measurements, total chlorophyll, and temperature), and microbiology (algae counts and bacterial populations).

Tilapia-shrimp polyculture has been adopted and adapted in many of the shrimp farming regions of the world and we hope to better define the methodologies and provide guidance as to which practices seem to be most beneficial.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## TESTING THREE STYLES OF TILAPIA-SHRIMP POLYCULTURE IN TABASCO, MEXICO

*Twelfth Work Plan, Production System Design and Integration 8 (12PSD8)*  
*Abstract*

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### ABSTRACT

The tilapia–shrimp polyculture project in Tabasco has been slowed by extensive renovations to the facility operated by the Universidad Juárez Autónoma de Tabasco. Arrangements were made for stocking the ponds more than a year ago, but plumbing and pond dike repairs were hindered by equipment malfunctions and problems with sub-contractors operating heavy equipment. Students designed the exact experimental protocols and were trained at the school in operating procedures.

Specifically our plan is to compare three different polyculture styles, sequential (with tilapia in one pond, then water passing to shrimp pond), simultaneous (tilapia in cages in shrimp ponds), and simultaneous with tilapia loose in shrimp ponds. We will focus on production results (fish and shrimp yields and survivals, Feed Conversion Ratios, and cost benefits), water quality parameters (dissolved oxygen, secchi disk measurements, total chlorophyll, and temperatures), and microbiology (algae counts and bacterial populations).

We expect to compare our results with findings from similar trials conducted in the Philippines and an earlier trial from the west coast of Mexico. Tilapia–shrimp polyculture has been adopted and adapted in many of the shrimp-farming regions of the world and we hope to better define the methodologies and provide guidance as to which practices seem to be most beneficial.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## BROODSTOCK DEVELOPMENT OF AMAZONIAN FISHES

*Twelfth Work Plan, Indigenous Species Development 2 (12ISD2)  
Final Report*

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### ABSTRACT

Forty-two *Piaractus brachipomus* broodstock between 4.0 and 5.0 kg (males) and 4.1 and 5.9 kg (females) were stocked in three earthen ponds at the La Terraza Aquaculture Research Facility of the Universidad Nacional de Colombia (Meta, Colombia). Selection factors for male and female broodstock, respectively, were semen release with a slight pressure on abdomen, or the presence of an enlarged flaccid abdomen, enlarged-reddish genital papillae, and egg nucleus migration for eggs sampled by catheter. Broodstock were pit-tagged and randomly distributed in three 250-m<sup>2</sup> ponds (14 per pond: even sex distribution). Water quality was modified to simulate the three most representative Amazon ecosystems where these fish naturally inhabit to evaluate water quality as a fish reproduction-conditioning factor. The three treatments were: T<sub>1</sub>: pH = 6, high tannic acid (1.20 mg/L) and conductivity (197  $\mu$ s/cm); T<sub>2</sub>: pH = 7, low tannic acid (0.42 mg/L) and conductivity (157  $\mu$ s/cm); and T<sub>3</sub>: pH = 8, low tannic acid (0.14 mg/L) and conductivity (131  $\mu$ s/cm). All treatments were characterized by having low alkalinities (<34 mg/L) and hardness (<62 mg/L). Temperature, D.O., pH, and conductivity were monitored three times per day (07:00, 12:00 and 17:00); CO<sub>2</sub> and ammonia were monitored once daily (07:00); and hardness, alkalinity, tannic acid, ammonium, nitrite, nitrate, sulphate, sulphite, and phosphate were monitored once weekly. After 33 days, fish were induced to spawn by hormonal injections (carp pituitary extract) and eggs from each female per treatment were fertilized with sperm from a separate male from the same treatment. Egg volume was calculated and eggs were incubated separately for each treatment to evaluate fertilization and hatching percentage. Blood samples were taken at the time of hormonal injection from at least four fish of each sex per treatment. Blood samples were collected for hematocrit evaluation and remaining blood was immediately frozen for later hemoglobin and steroid hormone (testosterone and estradiol-17 $\beta$ ) analysis. Two males and two females per treatment were anesthetized with MS-222 and euthanized to verify gonadal development macroscopically and to determine gonadosomatic index (GSI). Additionally, gonad portions were fixed in 6% buffered formalin for histological analysis. Spawning success was considered as the final indicators of treatment effect. Survival was 100% for the T<sub>2</sub> and T<sub>3</sub> treatments, while it was 42.8% for the T<sub>1</sub> treatment (three males and three females died of hypoxia). Individual females from the T<sub>1</sub> treatment presented the best macroscopic gonad maturation characteristics, as well as all males ( $n=4$ ) from the same treatment released sperm upon a slight abdominal pressure. Similarly, fertilization rates were 74.3% for the same treatment and GSI values were relatively high for T<sub>1</sub> males (0.29–0.41) and very high for the T<sub>1</sub> females (13.8–20.6). No fertilization rates were calculated for the other two treatments (T<sub>2</sub> and T<sub>3</sub>) since no gamete product were concurrently available from either gender. Further, GSI values were low for both T<sub>2</sub> males (0.09–0.10) and T<sub>3</sub> males (0.10–0.11) and relatively high for T<sub>2</sub> females (4.56–9.16) and T<sub>3</sub> females (6.37–8.62).

INTRODUCTION

Native species aquaculture has been expanding in the Amazon region in recent years. *Colossoma macropomum* (commonly called black pacu in English, gamitana in Peru, cachama negra in Colombia, cachama in Venezuela and Ecuador, and tambaqui in Brazil) and *Piaractus brachyomus* (commonly called red-bellied pacu in English, paco in Peru, cachama blanca in Colombia, morocoto in Venezuela and Ecuador, and pirapitinga in Brazil) are native to the Amazon Basin and possess many characteristics suitable for aquaculture. Both species are in high demand and bring a high price at the marketplace.

Fish is a major part of the diet of Amazon communities (Brazil and Peru; Eckmann, 1983). Advancements in the aquaculture of certain species in the region will maintain the supply for consumption without over fishing natural populations and, in effect, promote the utilization and conservation of wild stocks in the Amazon rainforest. Studies on propagation of *Colossoma macropomum* and *Piaractus brachyomus* (frugivorous fish) are important because the commercial fisheries overexploits these frugivorous fishes, thus the population is severely affected as the larger sizes of *Colossoma macropomum* and *Piaractus brachyomus* are sold at increasingly higher prices. There is increasing pressure in the conversion of the floodplains to rice paddies and cattle pastures (deforestation; Achard et al., 2002). Viable aquaculture may prevent this trend. This study could boost the interests in promoting the aquaculture of these indigenous species by providing information on their reproductive requirements. The objective of this study was to understand the effects that certain water quality parameters (pH, tannic acid and conductivity), from three very representative Amazon basin ecosystems, might have on gonadal steroidogenesis during final maturation of red-bellied pacu *Piaractus brachyomus*.

MATERIALS AND METHODS

**Objective 1. Improvement of the quality of progenies by manipulating water quality for *Piaractus sp.***

Forty-two mature red-bellied pacu *Piaractus brachyomus* (Table 1) between 4.0 and 5.0 kg for males ( $T_1 = 4,570 \pm 384$ ,  $T_2 = 4,318 \pm 244$  and  $T_3 = 4,321 \pm 259$  g) and 4.1 and 5.9 kg for females ( $T_1 = 4,768 \pm 716$ ,  $T_2 = 4,761 \pm 326$  and  $T_3 = 4,704 \pm 253$  g), were PIT-tagged (PIT-tags, Biosonic, Seattle, WA, USA) and randomly distributed in three 250-m<sup>2</sup> earthen ponds (14 per pond: even sex distribution) at the La Terraza Aquaculture Research Facili-

ty of the Universidad Nacional de Colombia (Meta, Colombia). Selection factors for male and female broodstock, respectively, were semen release with a slight pressure on abdomen, or the presence of an enlarged flaccid abdomen, enlarged-reddish genital papillae, and egg nucleus migration for eggs sampled by catheter. Water quality was modified to simulate the three most representative Amazon ecosystems where these fish naturally inhabit to evaluate water quality as a fish reproduction-conditioning factor. The three treatments were:  $T_1$ : pH = 6, high tannic acid (1.20 mg/L) and conductivity (197  $\mu$ s/cm);  $T_2$ : pH = 7, low tannic acid (0.42 mg/L) and conductivity (157  $\mu$ s/cm); and  $T_3$ : pH = 8, low tannic acid (0.14 mg/L) and conductivity (131  $\mu$ s/cm). All treatments were characterized by having low alkalinities (<34 mg/L) and hardness (<62 mg/L). Temperature, D.O., pH, and conductivity were monitored three times per day (07:00; 12:00 and 17:00) using a multiparameter YSI® 556 probe. CO<sub>2</sub> and ammonia were monitored once daily (07:00), and hardness, alkalinity, tannic acid, ammonium, nitrite, nitrate, sulphate, sulphite, and phosphate were monitored once weekly using a Hach® FF2 water quality kit with a portable spectrophotometer Hach® DR-850.

After 33 days, fish were induced to spawn by hormonal injections (carp pituitary extract) and eggs from each female per treatment were fertilized with sperm from a separate male from the same treatment. Egg volume was calculated and eggs were incubated separately for each treatment to evaluate fertilization and hatching percentage. Blood samples were taken at the time of hormonal injection from at least four fish of each sex per treatment. Blood samples were collected from each specimen from the caudal fin with a heparized syringe for hematocrit evaluation, hemoglobin and steroid hormone (testosterone and estradiol-17 $\beta$ ) analysis. Blood was centrifuged at 1500 rpm for 15 min and the plasma was stored at -20°C until assayed. Two males and two females per treatment were anesthetized with MS-222 and euthanized to verify gonadal development macroscopically and to determine gonadosomatic index (GSI). Additionally, gonad portions were fixed in 6% buffered formalin for histological analysis. Spawning success was considered as the final indicators of treatment effect.

The plasma concentrations of steroids (testosterone and estradiol-17 $\beta$ ) were measured by radioimmunoassay similar to those used previously (Ottobre et al. 1989) following ethyl-ether extraction. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 13.0. Data on growth performance and survival was subjected to one-way analysis of variance (ANOVA) followed by a comparison of

Table 1. Biometric data means for red bellied pacu *Piaractus brachyomus* broodstock subjected to three ( $T_1$ = Low pH, high tannic acid and high conductivity;  $T_2$ = Medium pH, medium tannic acid and medium conductivity; and  $T_3$ = high pH, low tannic acid and low conductivity) representative aquatic ecosystems in the Amazon basin

Treatment	Male			Female		
	Weight (g)	Total length (cm)	Standard length (cm)	Weight (g)	Total length (cm)	Standard length (cm)
$T_1$	4457 ± 384	53.5 ± 1.8	48.5 ± 1.6	4768 ± 716	54.8 ± 3.0	49.3 ± 3.2
$T_2$	4318 ± 244	52.9 ± 2.2	47.8 ± 1.8	4761 ± 326	54.5 ± 3.6	49.2 ± 3.5
$T_3$	4321 ± 259	52.9 ± 1.9	48.6 ± 1.6	4704 ± 253	54.7 ± 1.8	50.4 ± 1.6

means using the Least Significant Difference (LSD) Test (Steel and Torrie, 1980). Normality and homogeneity of variance tests was performed on raw data. Sample distributions violating assumptions were log-transformed before analysis. All differences were regarded as significant at  $P < 0.05$ .

## RESULTS

### Objective 1. Improvement of the quality of progenies by manipulating water quality for *Piaractus sp.*

Female individuals from the T<sub>1</sub> treatment presented the best macroscopic gonad maturation characteristics among all treatments. A single female from each treatment spawned (T<sub>1</sub> = 6,150 ml, T<sub>2</sub> = 775 ml and T<sub>3</sub> = 175 ml of hydrated eggs). In contrast, all remaining males ( $n=4$ ) from T<sub>1</sub> released sperm upon a slight abdominal pressure. Fertilization rates were 74.3% with a 91.0% hatching rate with a high egg quality (embryonic eyed-stage) for the same treatment (T<sub>1</sub>). No fertilization rates were calculated for the other two treatments since no gamete products were concurrently available from both genders.

GSI values (Table 2) were relatively high for T<sub>1</sub> males ( $0.35 \pm 0.05$ ) and very high for the T<sub>1</sub> females ( $17.02 \pm 2.97$ ) compared to the other two treatments (T<sub>2</sub> =  $0.11 \pm 0.01$  and T<sub>3</sub> =  $0.10 \pm 0.01$  in males, and T<sub>2</sub> =  $6.86 \pm 3.25$  and T<sub>3</sub> =  $7.50 \pm 1.59$  in females).

The precursor plasma sex steroid testosterone (Table 2) was not significantly ( $P > 0.05$ ) different in all treatments in males (T<sub>1</sub> =  $234 \pm 366$ , T<sub>2</sub> =  $1,561 \pm 1379$  and T<sub>3</sub> =  $1,661 \pm 416$  pg.mL<sup>-1</sup>) or females (T<sub>1</sub> =  $870 \pm 1,437$ , T<sub>2</sub> =  $549 \pm 993$  and T<sub>3</sub> =  $119 \pm 131$  pg.mL<sup>-1</sup>). In contrast, estradiol-17<sub>β</sub> plasma sex steroid concentration was significantly ( $P < 0.05$ ) higher in females than in males in all treatments (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>). Estradiol-17<sub>β</sub> was significantly ( $P < 0.05$ ) higher in T<sub>1</sub> females ( $643.4 \pm 183.0$  pg.mL<sup>-1</sup>) but lower in T<sub>1</sub> males ( $6.8 \pm 1.9$  pg.mL<sup>-1</sup>) compared to T<sub>2</sub> ( $11.2 \pm 2.7$  pg.mL<sup>-1</sup>) and T<sub>3</sub> ( $11.4 \pm 5.2$  pg.mL<sup>-1</sup>) males or T<sub>2</sub> ( $338.4 \pm 135.1$  pg.mL<sup>-1</sup>) and T<sub>3</sub> females ( $235.8 \pm 117.8$  pg.mL<sup>-1</sup>).

The stress index hemoglobin (Table 2) was significantly ( $P < 0.05$ ) lower in T<sub>1</sub> for both males ( $12.3 \pm 0.1$  g.dL<sup>-1</sup>) and females ( $12.2 \pm 0.2$  g.dL<sup>-1</sup>), followed by both T<sub>2</sub> males ( $12.5 \pm 0.2$  g.dL<sup>-1</sup>) and females ( $12.4 \pm 0.1$  g.dL<sup>-1</sup>), and T<sub>3</sub> males ( $13.0 \pm 0.2$  g.dL<sup>-1</sup>) and females ( $12.5 \pm 0.3$  g.dL<sup>-1</sup>). Furthermore, hematocrit, the other stress index indicator, was also significantly ( $P < 0.05$ ) lower for T<sub>1</sub> males ( $31 \pm 1$  mm) and females ( $29 \pm 2$  mm) compared to T<sub>2</sub> males ( $33 \pm 1$  mm) and females ( $31 \pm 1$  mm), and T<sub>3</sub> males ( $35 \pm 1$  mm) and females ( $33 \pm 2$  mm).

Survival was 100% for the T<sub>2</sub> and T<sub>3</sub> treatments while it was 42.8% for the T<sub>1</sub> treatment (3 males and 3 females died of hypoxia, D.O near 1.0 mg.L<sup>-1</sup>).

All treatments were characterized by having low alkalinities (<34 mg/L) and hardness (<62 mg/L) (Table 3). Temperature, pH, D.O., conductivity, CO<sub>2</sub>, ammonium, nitrite, nitrate, alkalinity, hardness and sulfur were within permissible levels for *P. brachyomus* reproduction.

## DISCUSSION

Environmental stimuli such as pH, dissolved oxygen, temperature, and photoperiod have been documented to affect reproduction of teleost fishes through the stimulation of the brain through sensory organs (eyes, olfactory and pineal gland) (Carolsfeld, 1989; Cheppalla et al., 1996; Gazola et al., 1996; Dabrowski et al., 2001, in Kohler et al., 2001). Dabrowski et al. (2001) suggested that blood plasma steroid profiles in *C. macropomum* are indicative for gonadosomatic indices of both sexes and can be critical in determining the time and magnitude of hormonal intervention.

In this experiment, both genders in treatment T<sub>1</sub> presented the best macroscopic gonad maturation characteristics and were the only group that released eggs and milt, concomitantly. These results were confirmed by the GSI values (Table 2), which were significantly ( $P < 0.05$ ) higher in males and very

Table 2. Plasma sex steroid hormones, gonadosomatic index (GSI), and stress index means in red bellied pacu *Piaractus brachyomus* subjected to three (T1= Low pH, high tannic acid and high conductivity; T2= Medium pH, medium tannic acid and medium conductivity; and T3= high pH, low tannic acid and low conductivity) representative aquatic ecosystems in the Amazon basin. Means within the same column with different letters are significantly different ( $P < 0.05$ ).

Treatment	Plasma Sex Steroids		GSI	Stress Indexes	
	Testosterone (pg.mL <sup>-1</sup> )	Estradiol-17 <sub>β</sub> (pg.mL <sup>-1</sup> )		Hemoglobin (g.dL <sup>-1</sup> )	Hematocrit (mm)
<b>MALES</b>					
T <sub>1</sub>	234 ± 366 <sup>a</sup>	6.8 ± 1.9 <sup>a</sup>	0.35 ± 0.05 <sup>a</sup>	12.3 ± 0.1 <sup>a</sup>	31 ± 1 <sup>a</sup>
T <sub>2</sub>	1561 ± 1379 <sup>a</sup>	11.2 ± 2.7 <sup>b</sup>	0.11 ± 0.01 <sup>b</sup>	12.5 ± 0.2 <sup>b</sup>	33 ± 1 <sup>b</sup>
T <sub>3</sub>	1661 ± 416 <sup>a</sup>	11.4 ± 5.2 <sup>b</sup>	0.10 ± 0.01 <sup>b</sup>	13.0 ± 0.2 <sup>c</sup>	35 ± 1 <sup>c</sup>
<b>FEMALES</b>					
T <sub>1</sub>	870 ± 1437 <sup>a</sup>	643.4 ± 183.0 <sup>c</sup>	17.02 ± 2.97 <sup>c</sup>	12.2 ± 0.2 <sup>d</sup>	29 ± 2 <sup>d</sup>
T <sub>2</sub>	549 ± 993 <sup>a</sup>	338.4 ± 135.1 <sup>d</sup>	6.86 ± 3.25 <sup>d</sup>	12.4 ± 0.1 <sup>e</sup>	31 ± 1 <sup>e</sup>
T <sub>3</sub>	119 ± 131 <sup>a</sup>	235.8 ± 117.8 <sup>d</sup>	7.50 ± 1.59 <sup>d</sup>	12.5 ± 0.3 <sup>e</sup>	33 ± 2 <sup>f</sup>



## INDIGENOUS SPECIES DEVELOPMENT

Table 3. Water Quality parameters for red bellied pacu *Piaractus brachyomus* broodstock subjected to three ( $T_1$ = Low pH, high tannic acid and high conductivity;  $T_2$ = Medium pH, medium tannic acid and medium conductivity; and  $T_3$ = high pH, low tannic acid and low conductivity) representative aquatic ecosystems in the Amazon basin

Treatment	pH	Temp. (°C)	Tannic Acid (mg.L <sup>-1</sup> )	D.O. (mg.L <sup>-1</sup> )	Conductivity ( $\mu$ S.cm <sup>-1</sup> )	CO <sub>2</sub> (mg.L <sup>-1</sup> )	NH <sub>4</sub> -N (mg.L <sup>-1</sup> )
$T_1$	6.0 ± 0.5	27.2 ± 1.4	1.2 ± 0.5	5.2 ± 1.9	197.4 ± 107.4	2.8 ± 0.6	0.001 ± 0.0001
$T_2$	6.9 ± 0.3	27.4 ± 1.4	0.4 ± 0.1	5.9 ± 1.4	157.0 ± 76.1	2.3 ± 0.5	0.001 ± 0.002
$T_3$	8.0 ± 0.2	27.1 ± 1.4	0.2 ± 0.2	7.0 ± 1.2	130.6 ± 33.4	1.6 ± 0.3	0.007 ± 0.035
	NO <sub>2</sub> -N (mg.L <sup>-1</sup> )	NO <sub>3</sub> -N (mg.L <sup>-1</sup> )	Hardness (mg.L <sup>-1</sup> )	Alkalinity (mg.L <sup>-1</sup> )	SO <sub>4</sub> (mg.L <sup>-1</sup> )	S (mg L <sup>-1</sup> )	
$T_1$	0.4 ± 0.3	0.8 ± 0.5	60	33	3.4 ± 2.3	0.008 ± 0.004	
$T_2$	0.4 ± 0.2	0.3 ± 0.3	62	34	2.8 ± 2.2	0.006 ± 0.005	
$T_3$	0.2 ± 0.2	0.8 ± 0.6	41	24	1.2 ± 1.6	0.002 ± 0.004	

high in  $T_1$  females. The plasma sex hormone estradiol-17 $\alpha$  was significantly ( $P < 0.05$ ) lower in  $T_1$  males and very high for females compared to  $T_2$  and  $T_3$  males and females. Similarly, GSI was greater in  $T_1$  males and females than in  $T_2$  or  $T_3$ . The combination of water quality parameters in  $T_1$  (pH, tannic acid, conductivity, albeit a temporal 2-d near hypoxic condition prevailed causing some mortalities) might have been the environmental triggering factors that increased testosterone plasma sex steroid concentration and consequently estradiol-17 $\alpha$ .

### CONCLUSIONS

Both genders in  $T_1$  (low pH, high tannic acid and high conductivity) presented the best macroscopic gonad maturation characteristics and this was the only treatment group to release eggs and milt, concomitantly.

The above conclusion is further supported by GSI values which were significantly ( $P < 0.05$ ) higher in  $T_1$  males and females and by the plasma sex steroid hormone estradiol-17 $\alpha$ , which was significantly ( $P < 0.05$ ) higher in  $T_1$  females than  $T_2$  or  $T_3$  females.

The combination of water quality parameters in  $T_1$  might have been the environmental triggering factors that increased testosterone plasma sex steroid concentration and consequently estradiol-17 $\alpha$ .

### ANTICIPATED BENEFITS

The study investigated key aspects of the reproductive biology of *Piaractus sp.*, through our collaborative effort with Colombian investigators in order to improve or develop sustainable aquaculture technology for these species. The main beneficiaries of this research are fish producers in the Amazon region. Development of the technology of intensive growth of these species and stocking 4–6 week-old juveniles will dramatically

increase their survival and efficiency of production. Of further importance, this study has also contributed towards institutional strengthening by providing training on various aspects of fish nutrition and reproduction for staff of the Universidad Nacional de Colombia (Instituto de Investigaciones IMANI), Instituto de Desenvolvimento do Amazonas (IDAM-Benjamin Constant), and Instituto Amazónico de Investigaciones Científicas-SINCHI (Programa de Ecosistemas Acuáticos, Colombia).

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### LITERATURE CITED

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## INCORPORATION OF THE NATIVE CICHLID (*PETENIA SPLENDIDA*) INTO SUSTAINABLE AQUACULTURE: REPRODUCTION SYSTEMS, NUTRIENT REQUIREMENTS, AND FEEDING STRATEGIES

*Twelfth Work Plan, Indigenous Species Development 3 (12ISD3)  
Abstract*

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### ABSTRACT

The first steps for a successful aquaculture program are to obtain spawnings systematically and produce high-quality eggs and healthy fry. To reach this point, it is necessary to obtain a high-quality batch of adults and to select for the most desirable traits to be expressed in subsequent generations. All of this is required to develop aquaculture programs capable of supporting medium-to-large-scale facilities. For many native cichlids, these aspects have not been evaluated, and because of the introduction of non-native species (i.e., tilapia) that have high reproduction, growth rates, and survival, their potential has been ignored. In southeastern Mexico, a growing number of fish producers are requesting the development of alternative culture techniques that involve native species. Since the early 1970s, the only species that has been available in this region is the Nile tilapia and little effort has been devoted to developing any alternatives. The culture of native species of fish is important from an economic and conservation standpoint at a time when the local and foreign demand has imposed great pressures on their natural populations. In this study, we have conducted experiments to evaluate the reproductive potential and growth capacity of *Petenia splendida* under diets that contain vegetable meals instead of fish meals. Three experiments were conducted to determine reproductive performance and fry survival. In Experiment 1, parental sex ratios were evaluated. Our results indicate that the 2:1 female:male ratio resulted in the highest production of eggs and larvae. In Experiment 2, fecundity and hatching success were evaluated. Our data indicate that fecundity in all spawnings was 100%. Hatching rate was higher than 95% in all cases. In Experiment 3, we evaluated the effect of stocking density on growth and survival of *P. splendida* fry. Results have shown that a density of 10 fry/L resulted in similar growth and survival compared to densities of 0.5, 1, and 5 fry/L, but higher than at a density of 20 fry/L. However, fry survival decreased as density increased. Survival for the densities of 0.5, 1, and 5 fry/L were 100, 98, and 96%, respectively, while densities of 10 and 20 fry/L had 89 and 68% survival. Three more experiments were conducted to evaluate the substitution of vegetable meal for fish meal at different life stages of *P. splendida*. In Experiment 4, we found that the best growth and survival was obtained when fry were fed with diets that had 25 and 50% of fish meal replaced with wheat gluten, compared with the other experimental diets (0, 75, and 100% wheat gluten). In Experiment 5, we observed the same results with juveniles, indicating that 25 and 50% replacement of fish meal with wheat gluten is feasible. Similar results were obtained in Experiment 6, where 25 and 50% replacement of fish meal with vegetable meal resulted in the highest growth and survival.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## BROODSTOCK DEVELOPMENT AND LARVAL FEEDING OF AMAZONIAN FISHES

*Twelfth Work Plan, Indigenous Species Development 4 (12ISD4)  
Final Report*

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### ABSTRACT

We successfully reproduced South American catfish (surubim, *Pseudoplatystoma* sp.) raised in captivity in the aquaculture facility at the Ohio State University, Columbus. One female spawned after receiving two doses of carp pituitary extract (0.5 and 5 mg/kg) at 11-h intervals. Eggs size was  $0.62 \pm 0.09$  mm in diameter. Two males released sperm and were used in fertilization. Sperm concentrations were  $24 \times 10^9$  and  $15.5 \times 10^9$  spermatozoa/ml in male 1 and male 2, respectively, and viability was confirmed after activation in 0.3% sodium chloride. Survival of embryos at 9 h after fertilization were 44% and 23% for male 1 and male 2, respectively. Embryos hatched 15 h after fertilization. Larvae were  $3.53 \pm 0.09$  mm in length at hatching. Larvae accepted *Artemia* nauplii two days after hatching. Nine days after hatching, larvae were provided with formulated dry diets and the transition was successful. Fifteen-day-old larvae were used in feeding experiments comparing live feeds (*Tubifex* sp. and *Artemia* nauplii), commercial, and semi-purified (casein and/or peptide-based) diets. Juveniles of 0.98 g individual weight were used to delineate protein/lipid optimum ratio in surubim. Nine casein-gelatin based diets with different protein/lipid levels were formulated. Protein levels were 40, 45 and 50%, and lipid levels were 12, 16 and 20%. Six weeks after the first feeding, survival averaged  $57.8 \pm 10.0\%$  among the treatments with no significant differences. Final individual weights did not differ between the dietary treatments, and the average weight gain was  $2124.2 \pm 295.7\%$ . Significant differences were found in trypsin activity in juvenile surubim with a trend toward an increase of activity corresponding to growth rate. Results of this study demonstrate for the first time that surubim raised in captivity can be reproduced and provide critical information on feeding procedures for larvae and juveniles of this species in controlled-intensive conditions.

### INTRODUCTION

South American catfishes (e.g., *Pseudoplatystoma coruscans*, *P. fasciatum* and *P. tigrinum*) are potentially important species for commercial production in South America (Kossowski, 1996; Campos, 2004). In Peru, spawning of *P. fasciatum* and *P. tigrinum* occurs in February–March (Alcantara, F., IAAP, Iquitos, personal communication), in Brazil in January (Leonardo et al., 2004). Final maturation and ovulation were achieved in several catfish species from South America using carp pituitary extracts or pituitary hormones (Cardoso et al., 1995; Kossowski, 1996). However, to the best of our knowledge, no information is available on the profiles of plasma sex steroids in *Pseudoplatystoma* species and we could possibly use this information to synchronize ovulation and spermiation in surubims as earlier described in other Amazonian fish (Dabrowski et al., 1996).

Annual changes in blood plasma steroids as well as the surge of maturational hormones preceding spermiation and ovulation can contribute to a better understanding of the dynamics of gonadal steroidogenesis. Moreover, such information will be useful in the development and standardization of breeding techniques through the use of natural and/or synthetic hormones. Our preliminary data indicated that the level of estradiol-17 $\beta$  and testosterone in females of *P. fasciatum* raised in a pond at the Instituto de Investigaciones de la Amazonia Peruana (IAAP, Iquitos, Peru) in March averaged  $0.35 \pm 0.2$  ng/ml and  $3.18 \pm 2.5$  ng/ml ( $n=4$ ).

The objectives of this study were (1) to determine gametogenesis and differentiation of ovary and testis in captive stock of

South American catfish, *Pseudoplatystoma* sp., (2) to determine changes in plasma sex steroid hormones during an annual cycle in *Pseudoplatystoma* sp., (3) to induce reproduction of *Pseudoplatystoma* sp., and (4) to assess blood plasma steroid response and gamete production (egg and sperm quality).

**MATERIALS AND METHODS**

South American catfish surubim (total length = 13 cm, weight = 12 g) were imported from a fish farm in Brazil (Fazenda Santa Rosa, Mato Grosso do Sul) in December 2001 (Lima et al., 2006), and flown to Madison, WI, USA where they were acclimated to laboratory conditions. Fish were then transferred to the aquaculture facility at The Ohio State University in March 2003. Since then, the fish have been monitored in terms of growth and sexual maturation. The majority of the fish were PIT-marked (passive integrated transponders; Destron Fearing Co., St Pail, MN) and 3–5 samplings were performed each year (weighing, sexing, blood withdrawn, fish sacrificed for gonad histology).

In March 2005, fish were transferred to larger tanks (200 L) where the 10–12 individuals were raised (biomass 12.5 kg / tank, water flow 6 L / min). Fish were fed pelleted feed (BioDiet Brood, Bio-Oregon, Inc., 5 mm) 0.5–1% body weight / day.

On November 4, 2005, fish from each tank were anaesthetized in MS-222 (0.5 g/L) and weighted individually. Blood samples ( $n=22$ ) were taken from the caudal vessel into a heparinized syringe, kept on ice, and then centrifuged at 5500 rpm at 4°C. Plasma was collected and stored at -20°C for further steroid analysis. Feeding rates were readjusted after weighing to 0.5% body weight.

The plasma concentrations of steroids (testosterone, estradiol-17β, and 11-ketotestosterone) were measured using radioimmunoassay methods similar to those used previously (Ottobre et al., 1989; Dabrowski et al., 2003) following ethyl-ether extraction.

Ten fish from those subjected to blood sampling were sacrificed and the gonads and livers were removed and weighed. The gonadosomatic index (GSI) was calculated as:  $GSI = (\text{gonad weight} \times 100) / \text{total weight}$ . The hepatosomatic index (HSI) was calculated as:  $HSI = (\text{liver weight} \times 100) / \text{total weight}$ . The gonads were fixed in Bouin’s solution for histological examination. Gonad fragments fixed in Bouin’s solution were processed

according to histological routine techniques as described earlier (Rinchard et al., 2002). They were embedded in paraffin, sectioned at 6 μm and stained with haematoxylin / eosin.

On February 6, 2006, fish were checked for signs of maturity (release of sperm by gentle pressure of the abdomen in males and oocyte biopsy using a catheter in females). Sperm was collected from two males weighing 3,599 (male 1) and 3,521 (male 2) g, respectively. The oocytes that were collected from six females varied from 1,936 to 4,605 g in mass were fixed in Bouin’s solution. These females as well as males that produced sperm on February 6, and two other potential males, were then injected with carp pituitary extract (0.5 mg/kg). On February 13, 2006, the females that were previously injected were injected again with two doses of carp pituitary extract (0.5 and 5 mg/kg) at 11-h intervals. Then these fish were observed regularly after treatment in accordance with the description given for propagation of *P. fasciatum* (Leonardo et al., 2004).

On February 14, 2006, one female produced eggs. Sperm samples were collected from male 1 and 2. Sperm from each individual male was used to fertilize eggs sub-samples (2 g with 0.1 ml sperm). The remaining eggs were inseminated with a mixture of sperm. Temperature was 25°C at fertilization and ranged from 25 to 27°C during the incubation period. Embryo survival was counted 9 h after fertilization.

On February 17, following yolk sac absorption, larvae were transferred and distributed into 12 aquariums with a semi-recirculated system at approximately 120–150 larvae per tank. Larval size was  $5.15 \pm 0.42$  mm at that time. Temperature was adjusted to 28°C. Salinity was adjusted at 2 ppt by adding Instant Ocean (Aquarium Systems Inc., Mentor, OH, USA) salt to the system. Three groups of larvae were fed with bloodworms, nine groups were fed with newly hatched *Artemia* nauplii at 10:00 am right after stocking. Bloodworms (*Chironomus*), were a homogenate of frozen worms (Fish King Inc., Chicago, IL, USA) with water (1:1). Fish were kept in constant dark. On February 24, larvae were provided with a formulated, commercial food (Aglo Norse, 500–710 μm, Norway).

On March 30, juvenile surubim ( $0.98 \pm 0.01$  g) were distributed in 24 aquaria (40 L volume) in a semi-recirculated system at density of 18 fish per tank. Nine casein-gelatin based diets with different protein/lipid levels were formulated. Protein levels were 40, 45

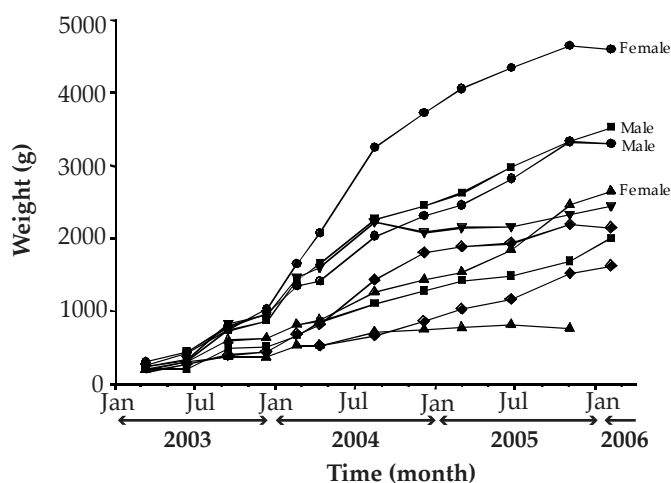


Figure 1: Individual growth of surubim

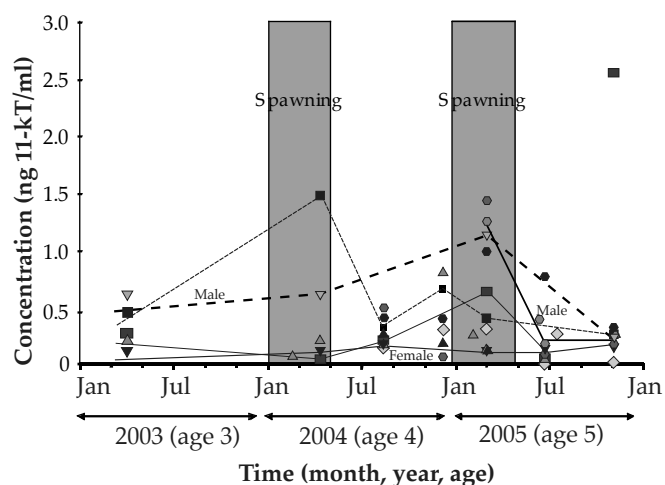


Figure 2: Sequence of “male steroid” in surubim

and 50%, and lipid levels were 12, 16 and 20% (soybean lecithin and cod liver oil). Fish were fed four times a day. Biomass was determined every two weeks. After the experiment ended (eight weeks), trypsin activity in the digestive tract was determined. Proteolytic enzymes were extracted from the whole digestive tract. Tissues were homogenized in 50 mM Tris-HCl buffer (pH 8.0) and following centrifugation (3000 x g), trypsin-like activity was analyzed as described earlier (Dabrowski and Ciereszko, 1994).

In a separate study, larval surubim (*P. fasciatus*) received from UNESP, Jaboticabal, Brazil. Surubim were hatched on February 9, 2005 (Brazil) and were shipped overnight to Columbus, as two-day-old. On the arrival, significant mortality was noticed and signs of cannibalism were recorded. Fish were transferred to a recirculated system maintained at 24.6°C. Fish were offered freshly hatched *Artemia* nauplii on the evening of February 11 and then continuously until February 18 when the first feeding experiment began. Fish were divided into 24, 40-L tanks, at density 30 fish per tank. At the completion of the first experiment, fish were divided into four class sizes and all offered *Artemia* nauplii to bring their size to as uniform as possible. The second experiment continued for two weeks. Fish, at 15 individuals per tank, in triplicate per treatment, were offered similar formulations.

RESULTS

Individual growth of surubim is shown in Fig. 1. It is evident that a great variation in weight gains takes place among fish in captivity, however it was not related to fish gender.

Like in many other fish (Rinchard et al., 2001), surubim males showed highly significant increases in plasma “male” androgen, 11-kT, at the time corresponding to the spawning in their “native geographical zone”. In teleosts, this androgen reaches its maxima at the end of spermiogenesis, and declines soon after the initiation of spermiation. Indeed in both years, several fish that were later confirmed to be males, released sperm between January and March (Fig. 2).

In November 2005, oocyte diameter averaged 365 ± 35 µm varying from 306 to 421 µm (Fig. 3). GSI varied from 0.8 to 1.2%, averaging 1.0 ± 0.2%. HSI was 0.4 ± 0.1% ranging from 0.3 to 0.5% (Table 1). There was a negative correlation between

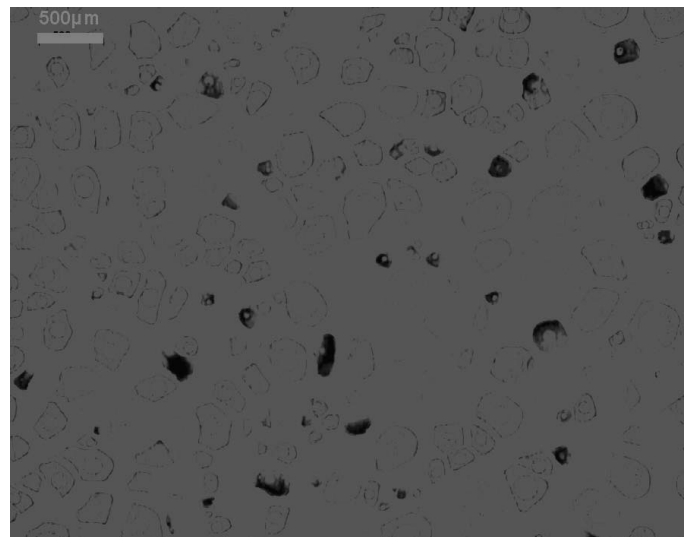


Figure 3: Ovary cross-section of a female catfish weighing 1737 g

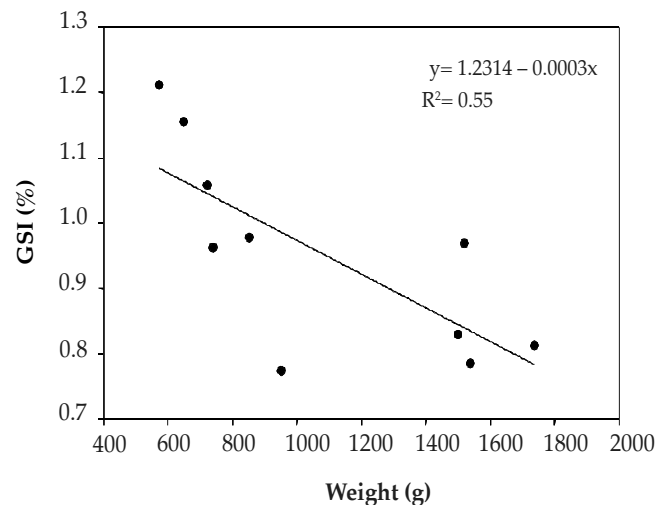


Figure 4: Relationship between fish mass and GSI

Table 1: GSI, oocyte diameter and HSI of catfish on November 4, 2005

Fish #	Body weight (g)	Gonad weight (g)	GSI (%)	Oocyte* diameter (µm)	Liver weight (g)	HSI (%)
1	740	7.1	1.0	383 ± 21	3.1	0.4
2	1737	14.1	0.8	332 ± 13	9.0	0.5
3	852	8.3	1.0	330 ± 29	3.3	0.4
4	572	6.9	1.2	347 ± 23	2.9	0.5
5	1538	12.1	0.8	306 ± 46	6.7	0.4
6	1500	12.4	0.8	378 ± 67	5.6	0.4
7	1520	14.7	1.0	421 ± 57	6.4	0.4
8	951	7.4	0.8	402 ± 39	3.1	0.3
9	649	7.5	1.2	360 ± 45	2.4	0.4
10	721	7.6	1.1	365 ± 43	3.0	0.4

\*Values mean ± SD

Table 2: Females attempted to spawn in 2006

Weight (g)	Time	Mean oocyte diameter (mm)	Eggs obtained (g)	Survival to hatch (%)
2064	February 14	0.62 ± 0.09	48	44 and 23*
2027	February 10	0.74 ± 0.06	121	No fertilization
4605	April 2	0.56 ± 0.08	Small amount	No fertilization
2190	February 6	0.30 ± 0.08	No eggs	N/A
3564	February 6	0.48 ± 0.12	No eggs	N/A
1936	February 6	0.31 ± 0.09	No eggs	N/A

\*Survival at 9 h is presented separately for 2 males

fish mass and GSI (Fig. 4). We also determined the oocyte diameter for female catfish sampled at different times since 2003. There was a statistically significant correlation between GSI and oocyte diameter (Fig. 5).

On February 6, 2006, sperm concentrations reached  $24 \times 10^9$  and  $15.5 \times 10^9$  spz/ml in male 1 and male 2, respectively. Oocytes measured for each female varied from  $0.30 \pm 0.08$  to  $0.74 \pm 0.06$  mm in diameter. On February 14, 2006, 9 hours after the second injection, 47.9 g of eggs (~114,500 eggs,  $0.62 \pm 0.09$  mm in diameter) were produced by a female weighing 2,064 g (Fig. 6, Table 2). Embryo survival 9 h after fertilization were 44 and 23% for male 1 and male 2, respectively (Fig. 7). Embryos hatched at 11:00 pm, 15 hours after fertilization. Larvae were  $3.53 \pm 0.09$  mm in length at hatching (Fig. 8). The characteristics of the female that we attempted to spawn are presented in Table 2. Two other females also spawned but fertilization failed.

On February 17, four hours after the first feeding larvae were checked under the microscope and *Artemia* nauplii were detected in the “presumptive stomach” already, but no bloodworms were accepted. Therefore, bloodworm-fed groups were switched to *Artemia* nauplii. Commercial food (AgloNorse, 500-710 μm; Evos, Norway) was accepted by the larvae on February 24, 2006.

In respect to the experiment with different protein/lipid levels provided as casein-gelatin based diets, six weeks after the first feeding, survival averaged  $57.8 \pm 10.0\%$  among the treatments with no significant differences. Final individual weight did not differ between the dietary treatments and the average weight gain was  $2124.2 \pm 295.7\%$  (Fig. 9). Trypsin activity in the digestive tract is presented in Fig. 10. There were significant differences in trypsin-like activity among dietary treatments and activity corresponds to weight gain. Furthermore, specific activities (expressed per fish weight) indicated that effect of dietary protein level on the activity of trypsin in fish depend on fish size and trend may be opposite at low and high protein levels.

We also report here for the first time the differences in diets acceptance, fish growth and diet utilization in surubim (*P. fasciatus*) at early stages of ontogeny (10 mm, total length) and in juveniles (25 mm). Fish offered *Artemia* nauplii overperformed those transitioned to live tubificid worms or two commercial diets both in terms of weight gains and survival. Fish offered

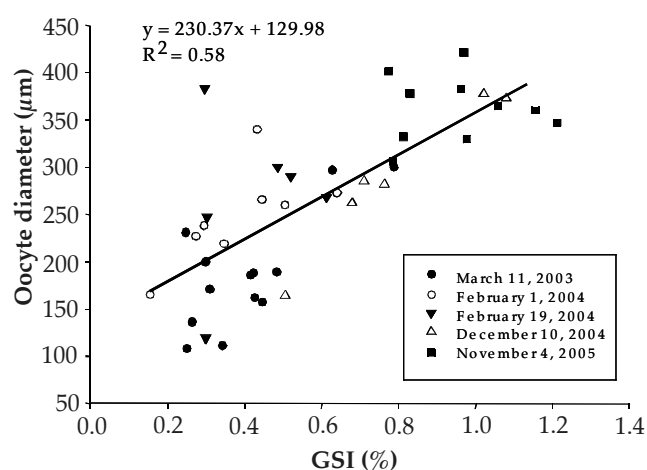


Figure 5: Relationship between oocyte diameter and GSI of surubim catfish sampled at different times.

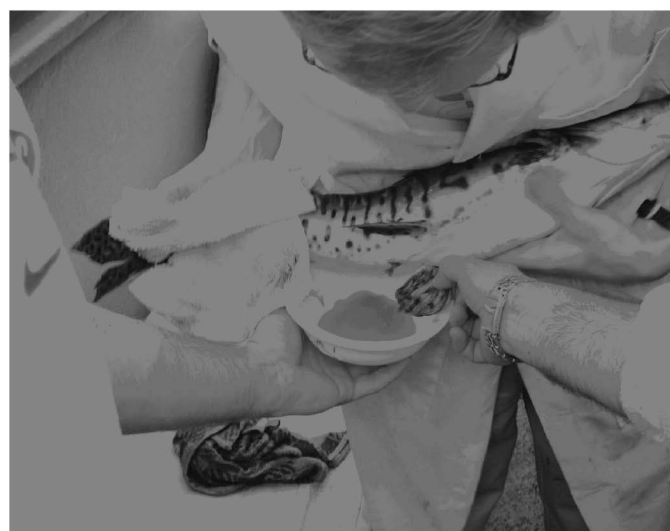


Figure 6: Spawning of surubim

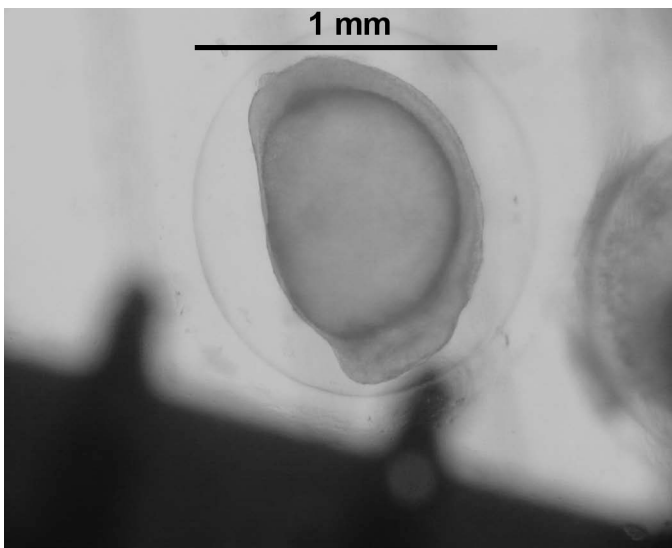


Figure 7: Embryonic development of surubim (9 h after fertilization)

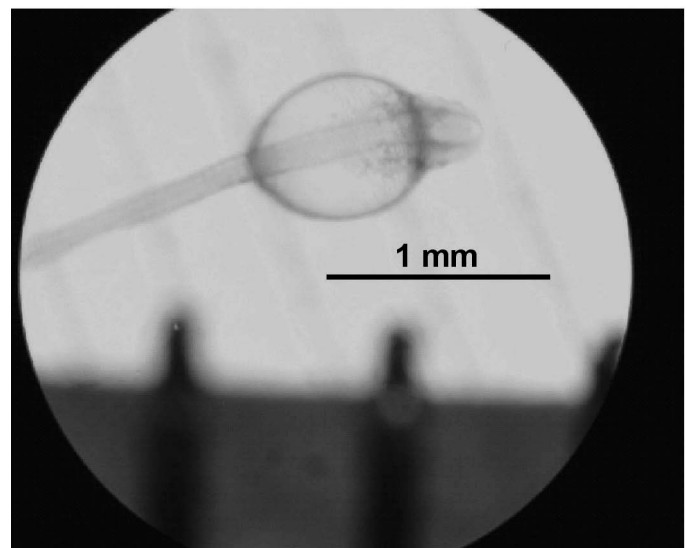


Figure 8: Newly hatched surubim larvae

semi-purified diets based on casein/gelatin or synthetic dipeptides (50% protein) accepted formulated feeds, gained weight, and in the case of peptide-based diet had an excellent survival (85%). Juvenile surubim (initial weight  $100.5 \pm 5.1$  mg) grew best when offered the marine larval diet (Aglo Norse), however, severe cannibalism was observed ( $42.2 \pm 13\%$ ).

### DISCUSSION

Growth of surubim in laboratory conditions was high. On the basis of the present results, the commercial diet used (Biodiet Brood, BioOregon, 46% protein, 13.5% lipid) seems adequate to promote growth of surubim broodstock.

Brito and Bazzoli (2003) reported GSI to be  $0.28 \pm 0.15\%$  and  $0.07 \pm 0.04\%$  in females and males, respectively in resting *P. coruscans* collected in the Sao Francisco River in Brazil. In contrast, these authors observed a significant increase of GSI when fish approached maturity ( $4.61\% \pm 2.10$  and  $1.97 \pm 0.48\%$ , in female and male, respectively). Garcia et al. (2001) obtained the maximum value of GSI for *P. fasciatum* and *P. tigrinum* in February as 2.8 and 1.1% (combined sexes), respectively. These fish were collected in the upper Amazon tributary, Rio Ucayali, Peru. In the present study, in fish in captivity, GSI averaged  $1.1 \pm 0.2\%$  on November 4, 2004 and reached  $\sim 2.5\%$  during spawning (February 14, 2006; based on the spawning female).

We have evidence that Amazonian catfish can be produced successfully in captivity in North America and the next generation of fish acclimated in the northern hemisphere can certainly become more amenable to intensive culture conditions. This also creates opportunities for an ornamental fish industry as public in the USA and Europe is concerned about conservation of the tropical fish species in the Amazon.

### ANTICIPATED BENEFITS

We achieved the first induced spawning of South American *Pseudoplatystoma* sp. on the North American continent. Furthermore, we accomplished spawning with fish maintained in captivity, in a recirculation system, under semi-controlled water temperature and light regime conditions. This is an

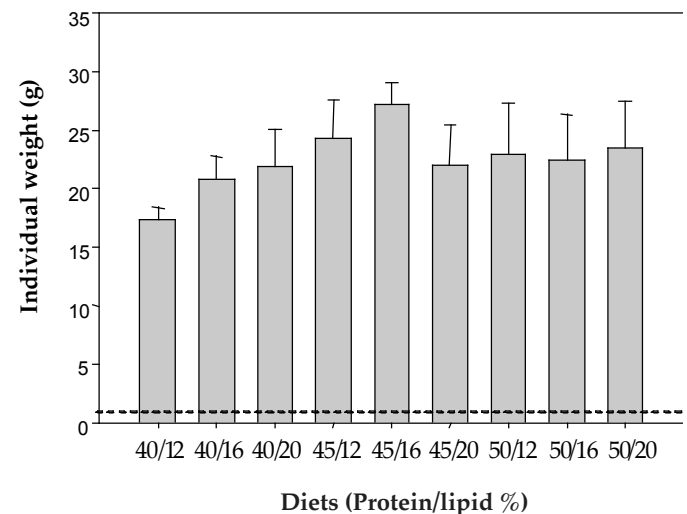


Figure 9: Growth response of juvenile surubim fed diets with different protein/lipid levels over eight week period.

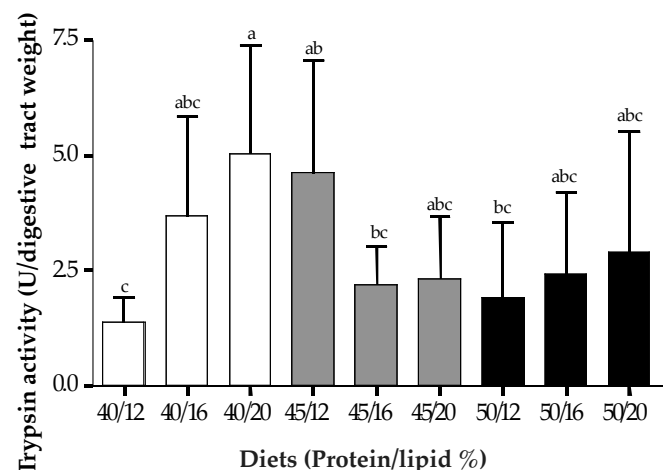


Figure 10: Trypsin activity in digestive tract of juvenile surubim fed diets with different protein/lipid levels over eight-week period



achievement in its own right. The information generated in this project, in regard to steroid profiles in the blood of surubim in captivity will be relevant for comparison to fish in natural or semi-natural (pond rearing) conditions in the Peruvian Amazon and other subtropical locations in South America where interest in surubim aquaculture is booming (Brazil, Colombia).

#### ACKNOWLEDGMENTS

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## POND DESIGN AND WATERSHED ANALYSES TRAINING

*Twelfth Work Plan, Water Quality and Availability 1 (12WQA1)*  
*Abstract*

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### ABSTRACT

In many parts of the world, including Central America, rainfall is highly variable throughout the year. This extreme variation in rainfall from month to month frustrates efforts to develop surface water as a viable alternative for water supply for aquaculture, irrigation, or other uses. Developing impoundments in regions with highly variable rainfall may lead to several times the volume of the pond effectively passing through the pond location and thus causing premature failure due to siltation. High runoff rates increase the risk of failure due to difficulties in achieving adequate spillway capacity.

The strategy advanced to address the variable rainfall-runoff while achieving useful water storage in ponds is to manage pond inflow by diverting most runoff into the pond during dry times and diverting most runoff away from the pond during wet months.

A simple rainfall-runoff catchment analysis model has been developed that can simulate monthly rainfall-runoff in small watersheds. The model runs on the Excel platform and can easily be implemented on any PC running Microsoft® Office. Twenty three inputs describe the catchment characteristics, pond area and depth, proposed pond spillway materials, and regional climatic variables. The user may also input a diversion factor for each month to simulation of diversion management strategies to assess impact on the pond feasibility. Using this information, the model computes runoff volume, pond volume, and spillway sizes. A scoring system may then be used to evaluate the suitability of the diversion management strategy. A scoring system ranging from 1 (unsustainable pond) to 5 (sustainable pond) was developed following trends in cumulative overflows and underflows. The model, with versions in English and Spanish, is available for public use.

A presentation series has been developed which consists of powerpoint presentations and a software CD. These presentations have been delivered in Nicaragua, Honduras, and the Dominican Republic in the past year.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## ELIMINATION OF METHYLTESTOSTERONE FROM INTENSIVE MASCULINIZATION SYSTEMS: USE OF ULTRAVIOLET IRRADIATION OF WATER

*Twelfth Work Plan, Water Quality and Availability 2 (12WQA2)  
Abstract*

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### ABSTRACT

Methyltestosterone (MT) is a light-sensitive hormone which is subject to photodegradation. The type of light most likely responsible for photodegradation is UV-B (wavelengths of 280–315 nm). Methyltestosterone absorbs UV light strongly at a wavelength of 254 nm, which is in the UV-C part of the spectrum (100–280 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. Little is known about the amount of exposure to UV needed to remove MT or 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. This study tested the hypothesis that MT could be eliminated from the water for intensive sex-inversion systems using UV sterilizers emitting a wavelength of 254 nm. Water samples were collected daily after hormonal treatment of tilapia fry at different times. MT was extracted by filtering 20 ml of water with Sep-Pak cartridges and MT content is currently being determined by radioimmunoassay at Oregon State University.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## ELIMINATION OF METHYLTESTOSTERONE FROM INTENSIVE MASCULINIZATION SYSTEMS: USE OF SOLAR IRRADIATION AND BACTERIAL DEGRADATION

*Twelfth Work Plan, Water Quality and Availability 3 (12WQA3)  
Abstract*

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### ABSTRACT

Methyltestosterone (MT) is a light-sensitive hormone which is subject to photodegradation. Speculation among farmers based on this characteristic has led to a general belief that MT can be degraded by exposing the water with the steroid to sunlight. In a previous study we developed a Recirculating Aquaculture System (RAS) to eliminate MT from aquaculture effluents in an intensive system for masculinizing tilapia fry at a large scale. In this system, the excess MT was eliminated from the water and the substrate by means of continuous filtration through activated charcoal filters. The RAS is economical, easily constructed, and is composed of a submersible pump, sediment trap, charcoal filter section, mechanical filter section, and a biological filter section. After the water leaves the RAS it returns to the tank through a perforated section of PVC pipe resulting in a "water curtain" which both aerates the water and exposes it to sunlight. Results from the study showed that although MT was eliminated from the water and accumulated in the charcoal of the RAS, water from control treatments (MT-treated water, but with no charcoal filters when passed through the RAS) also did not have detectable levels of MT. In another investigation, we demonstrated that exposure of MT-treated water to sunlight resulted in reduced levels of the compound but it was not completely eliminated. It is also known that some bacteria are capable of degrading steroids. From this information and results from the previous two investigations, we hypothesized that MT was being eliminated from control water by solar irradiation and/or bacterial degradation within the RAS. This study tested the hypothesis that MT could be eliminated from the water used in intensive sex-inversion systems using biological filtration and/or sunlight exposure. Two experiments were conducted and water samples were collected daily at different times after either simulating a feeding regime or adding a large dose of MT to the water. No fish were present in both experiments. MT was extracted by filtering 20 ml of water with Sep-Pak cartridges and MT content will be determined by radioimmunoassay at Oregon State University.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## ECOLOGICAL ASSESSMENT OF SELECTED SUB-WATERSHEDS OF THE NZOIA RIVER BASIN

*Twelfth Work Plan, Water Quality and Availability 4 (12WQA4)  
Abstract*

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### ABSTRACT

During the six months of the study, 73 macroinvertebrate taxa were encountered representing 13 orders and 51 families with a total abundance of 13,910. The major taxonomic groups encountered were Diptera, Ephemeroptera, Coleoptera, Oligochaeta, Trichoptera, Gastropoda, and Odonata representing 37%, 22%, 11%, 11%, 10%, 5%, and 2%, respectively. Other groups collected in small numbers include Hemiptera, Plecoptera, Hirudina, Crustacea, Arachnida, and Lepidoptera.

During the study, it was generally observed that abundance was lowest during the dry period. It increased progressively during the onset of the rainy season but started to decline during the peak and spates in July–August. However, the spates and heavy flows did not affect taxon richness although abundance was greatly affected. At this period, the river was characterized by large quantities of suspended matter and high sediment loads. Discharge also increased by more than ten times.

Non-recording rain gauges were installed in order to collect data on the amounts of precipitation that will help in predicting the quantities and rates of runoff generated during rain events. To facilitate in the collection of this data, we have trained research assistants to collect and record rainfall data. Data on suspended solids collected before the onset of rains will assist in making comparisons. Increased turbidity of Moiben River waters have been noted during rain events, with suspended solids recorded at greater than 20mg L<sup>-1</sup>. Hence the sampling process has been coupled with education of the community by the relevant authorities, targeting those cultivating close to the river, on best management practices to reduce soil erosion.

We have also collected and examined groundwater samples for contamination with atrazine and nitrates. Our preliminary results indicate higher levels of contamination at the start of farming activities. The extent and knowledge about use of pesticides and fertilizers are obtained by use of questionnaires. The possible effects on health of the inhabitants will be established using results from the questionnaires and medical records available at medical facilities.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## DETERMINATION OF HYDROLOGIC BASELINES FOR THE NZOIA RIVER BASIN

*Twelfth Work Plan, Water Quality and Availability 5 (12WQA5)  
Final Abstract*

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### ABSTRACT

A software suite consisting of GoogleEarth, GoldenGraphics Surfer, and UTS TKSolver was assembled and preliminarily evaluated for assessing the potential of soil erosion due to large-scale agricultural development, which can enter and damage the Nzoia River basin in Kenya. Based on comparisons of elevations obtained with handheld GPS units onsite and measurements made with GoogleEarth software, we determined elevation data, area measurements, and watershed cover assessments can be used to assess the relative potential for agricultural practices to contribute to sedimentation in streams. The Moore's Bridge subwatershed, located near Eldoret, Kenya on the Moiben River, was selected as the first to study. An extensive analysis of the components of the Universal Soil Loss Equation and the US Forest Service sediment delivery ratio method was made. Based on the fact that rainfall in the central to south African region is close to that in the US and that crops common to the US are in production, we feel confident that the US experience is applicable. Soils of the region are of the Ultisol and Oxisol classification, similar to soils in the southeast US. Using the Universal Soil Loss Equation coupled with the US Forest Service sediment delivery ratio method, we determined that topography could be easily mapped and assessments made of erosion potential. Preliminary results suggest that agricultural pollution is not a serious issue in this particular region.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## ASSESSMENT OF COASTAL AND MARINE AQUACULTURE DEVELOPMENT FOR LOW TROPHIC LEVEL SPECIES

*Twelfth Work Plan, Economic/Risk Assessment and Social Analysis 1 (12ERA1)*  
*Abstract*

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### ABSTRACT

The primary aim of this report is to provide the ACRSP an overview of the literature to-date regarding use of low trophic level species in near shore aquaculture development around the world. Additionally, the study will provide a detailed analysis of the literature and make specific recommendations regarding the prioritization of research needs as they relate to culture of low trophic level species in the near shore. This project was initiated 1 January 2005.

We focus our definition and research of low trophic level, near-shore aquaculture on aquaculture systems that we refer to as Low-Trophic Ecological Aquaculture in the Near shore, or LEAN. The principles defining LEAN were adopted from Costa-Pierce's philosophy of "ecological aquaculture" and applied to the near shore. LEAN systems 1) include any method of cultivating plant or animal species that requires minimal to no outside energy or food inputs to foster adequate growth; 2) occur in coastal estuaries, beaches, bays, inlets, or lagoons that can be easily managed for human benefit with little invested capital or equipment; 3) have minimal effects on the environment; and 4) can be integrated within local socio-economic and cultural structures.

The first section of this report will define and describe LEAN for the purposes of this study; the second section will provide an overview on the current status of LEAN in the near shore; the third section will detail the methodology used to search the literature; and the fourth section will detail the specific ecological, economic, social, political, and cultural issues relevant to LEAN development in the near shore region. In the fifth section of the report, the material presented in earlier sections will be synthesized and applied in the analysis of three case studies, each of which involves the use of LEAN for three distinct purposes: 1) as effluent treatment for high-intensity production systems, 2) for small-scale production of food and income, and 3) for enhancement of wild-capture fisheries.

The possibilities for LEAN systems are innumerable and routinely site-specific. The literature relating to this topic is immense and extremely diverse. LEAN systems occur worldwide with a myriad of species and in a multitude of forms that include monoculture, polyculture, and integrated culture. The literature on LEAN-related topics reflects its variability and ubiquity.

We conclude by presenting a conceptual model of the ideal LEAN project based upon the literature and case studies examined, and make four recommendations in which the ACRSP could pursue productive research on LEAN and promote effective LEAN project development. These four recommendations are to: 1) identify already existing practices that could be developed into more formalized LEAN systems in each ACRSP country; 2) perform in-depth case studies of established LEAN practices with a goal to better understand their failures and successes; 3) establish local educational and training programs that can instruct local peoples on LEAN development; and 4) financially support LEAN projects currently underway and establish long-term research projects at selected sites focusing on the interactions between LEAN systems and near-shore ecosystems.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## HYDRAULIC, WATER QUALITY AND SOCIAL ASSESSMENT OF THE NZOIA BASIN, KENYA

*Twelfth Work Plan, Economic/Risk Assessment 2 (12ERA2)  
Final Report*

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### ABSTRACT

Assessing risks and assets aids in understanding how livelihood strategies reflect changes in vulnerability. Mitigating risks is dependent on people's ability to pool and accumulate assets on either an individual basis or community-wide level. In Western Kenya, livelihood strategies along the Kapoet River and Moiben River reflect the role of financial, human, natural, physical, and social capital. Research objectives include identification of: 1) risks and assets and 2) the strategies used to manage and enhance the capital resources in the Kapoet and Moiben regions. Data were acquired in each location through participatory rural appraisal activities ( $n=4$ ), informant interviews ( $n=8$ ), household questionnaires ( $n=172$ ), and personal observation. Participatory risk ranking activities identified health issues, communication infrastructure, insecurity, water, markets, unemployment, and leadership as major concerns. Notable identified assets included local natural resources, churches, police posts, governmental extension services or representatives, non-governmental organizations and community-based organizations. Involvement in community self-help groups, altering farming strategies, and seeking outside assistance were strategies people used to help reduce risks and strengthen assets. Assisting communities in identifying opportunities for risk reduction and asset enhancement may contribute to more effective land management and poverty alleviation strategies.

### INTRODUCTION

Livelihood vulnerability is an emerging concept in the development sector and may assist in understanding livelihood management for mitigating risks and accumulating assets. At individual, household, or community scale, measuring livelihood vulnerability involves an assessment of actual and perceived risks and assets. "Livelihood comprises the capabilities, assets (including both material and social resources) and activities required for a means of living" (DFID, 2001:1). Assessing human vulnerabilities involves social capital, livelihood resilience, social protection, self-protection, and well being in order to identify resilience, resistance and exposure to risk (Cannon, 2000; Few, 2003). Risks include physical, biological, economic, and political variables that result in unfavorable livelihood conditions. Assets include the same variables as risks, but are viewed as favorable livelihood conditions. The International Livestock Research Institute (2004) is one development organization that identifies livelihood assets to include: financial, human, natural, physical, and social capital (Kristjanson et al., 2004). Financial capital consists of the availability of cash, cred-

it, and savings (Kristjanson et al., 2004). Human capital consists of knowledge, skills, labor, and health (Kristjanson et al., 2004). Natural capital includes resources found in land, air, water, wildlife, forests, and other natural resources (Kristjanson et al., 2004). Physical capital incorporates infrastructure features such as housing, water source, energy and communication (Kristjanson et al., 2004). Social capital comprises memberships, networks, institutions and access to influential people (Kristjanson et al., 2004). Financial, human, natural, physical, and social capital combined with the biophysical assets of location assist in the development of household and community strategies for addressing livelihood risks.

In Kenya, research has explored economic, human, natural, physical, and social capital to understand the relationships between livelihood strategies and vulnerability. As research seeks to understand what determines peoples' land use strategies, the relationships between culture and the environment have emerged as key elements for understanding land use activities



(Thomas-Slayter and Rocheleau, 1994). Previous research relied on biophysical models for predicting land use activities (Thomas-Slayter and Rocheleau, 1994). This livelihood approach to development research focuses on assets and household strategies, whereas vulnerability analysis emphasizes negative characteristics and focuses on risks (Cannon 2000; Few 2003). "The ways that people divide and share knowledge, access, use, and control in rural resource management reflects the social, political and economic context at the local and national level" (Thomas-Slayter and Rocheleau, 1994:6). "These factors influence the character and condition of the physical landscape as well as the roles of men and women as resource users, owners, managers and caretakers" (Thomas-Slayter and Rocheleau, 1994:6). Using the analysis of the relationship among gender, resources, and sustainable development there is now a focus on the resource users, their resiliency and flexibility to deal with risks (Thomas-Slayter and Rocheleau, 1994).

The concept of resilience within vulnerability analysis originates from ecological research where it defines a system's ability to recover after a disturbance and the system's ability to function, tolerate, or adapt to disturbances (Turner et al., 2003). Human vulnerability assessment also includes an assessment of a social system's resiliency to vulnerabilities in their ever-changing environments. Identifying people's ability to prepare for and recover from a disturbance, the extent of social networks and the choices people have about their exposure to risks help determine resiliency, which contribute to their overall livelihood vulnerability (Montz and Evans, 2001).

## METHODS AND MATERIALS

### Data Collection

Participatory Rural Appraisal (PRA) methodology enables communities to identify their own strengths and weaknesses and has the capability to empower them to engage in activities to reduce their known risks. PRA risk analysis was found to detect within-group heterogeneity of both exposure and severity of risks by Smith et al. (2000). This method has also proven useful to identify underrepresented issues (Smith et al. 2000). Holloway and Lindsey (1996) found PRA assessment useful in analyzing community vulnerability to risk, which actively allows the community to identify their perceived risks and capacity to cope with or prevent risks.

PRA assessments were carried out in two village/locations within each subwatershed (Moiben and Kapolet Rivers) in the upper highlands of the Nzoia basin. Data were collected through personal observation, key informant interviews, and PRA events including: transect walks ( $n=8$ ), problem rankings, resource ranking/mapping, and asset identification. Data on assets, resource limitations, and livelihood strategies were also captured through household interviews ( $n=172$ ), key informant interviews ( $n=8$ ), and personal observations.

Community PRA activities spanned four days in each location. The initial day covered a time-line of significant events in the last ten years and sketching a resource map; these activities were open to all community members. Separate meetings for men and women took place over the next two days. These meetings were used to complete risk identification and ranking activities. The final day involved resource and land use activity identification through transect walks of the community. To promote broader participation locally influential people were

identified by the research assistants and were given only a brief period to talk while encouraging other community members to speak. Demographic characteristics of the community meeting participants were unknown. During the gender-based community meetings, the participants as a group identified their risks. People were asked about their problems or issues of greatest concern to their livelihood, these risks were not asked in the context of agricultural production. Risks were assessed to determine the extent of each risk in terms of its affect on the entire community and then were ranked in descending order from most to least severe. During this process, ten individuals (note: only nine individuals were available in Munyaka's women's group) were chosen by random selection to individually rank the severity of each listed risk/problem by how it affected them personally.

### Data Analysis

PRA methodology allows researchers to gain information through active community participation, letting the community members express their assets, risks, and vulnerabilities to certain circumstances. Risk and asset analysis incorporated with PRA techniques allowed people to prioritize their risks and assets. Founded on PRA methodology, risks were assessed through a series of risk identification and ranking activities as reported in Smith et al. (2000) and replicated by Quinn et al. (2003). Smith et al. (2000) created an index system for severity and incidence for each identified risk. The severity index assigned a value of 1 to severe risks and 2 to less severe risks etc. (Smith et al., 2000). To normalize the degree of risk severity, the rankings were converted to an index scale where 1 had the highest degree, two had the lowest degree, and all other degrees were integers between (Smith et al., 2000). To calculate incidence, an index was made by proportion of respondents who identified the risk factor as, 0—no one was affected and 1—everyone was affected. This proportional incidence measurement captured how widespread the issue was within the community (Smith et al., 2000). To combine these indexes the following formula was used for each identified risk  $S_j = 1 + (r - 1)/(n - 1)$ , where  $S_j$  was the severity index value for each risk,  $r$  was the rank,  $n$  was the total number of risks identified and  $j$  was each specific respondent (Smith et al. 2000). The means of each risk identified were used to calculate the sample severity index (Smith et al., 2000). The risk index was calculated using  $R_j = I_j/S_j$ , where  $I_j$  was the incidence index value per respondent (Quinn et al., 2003). This process of risk identification and assigning weighted values for severity and incidence for each risk allowed risks to be more realistically weighted compared to regular ranking activities that occurred in the larger group activity. Individual risk index values were also calculated for each person selected, which used the risk index method of weighting risk incidence and rank. The means of the risk index values were then compared using independent samples Mann-Whitney test to detect for a significant difference at  $\alpha = .05$  level for gender, region, and gender differences within each village. All statistical data analyses were performed using SPSS version 12.0 (SPSS 2003).

Table 1. Participant Numbers

Location	Community Meeting	Men's Meeting	Women's Meeting
Munyaka	69	38	14
Sengwere	106	24	47
Meibeki	53	22	50
Barsombe	37	26	45

**RESULTS**

The numbers of participants at each meeting site are indicated in Table 1. The process of weighing risk severity and incidence helped distinguish heterogeneity within a community, especially since individual concerns can be suppressed in a larger community meeting. The risks identified during community meetings by all study sites included: disease and health issues, insecurity and illegal activities, poor communication resources, insufficient markets, limited farming knowledge and poor education (Table 2). Disease (malaria, upper respiratory tract infections, and diarrheal infections) and waterborne illnesses were common health concerns along with the inability to access adequate health facilities. Insecurity and illegal activities were concerns for all locations although each site had unique concerns such as cattle theft, burglaries, logging, charcoal production and breweries. Poor communication infrastructure was a problem in each site regardless of remoteness. Roads remained impassible during the rainy season, which limited access to markets, medical facilities, and sources of communication (e.g., telephones, bus stops, post offices). Concerns with markets in-

Table 2. Risk Identification- Community Meeting Summary

Identified Risks/Problems	Munyaka		Sengwere		Meibeki		Barsombe	
	Rj	rank	Rj	rank	Rj	rank	Rj	rank
Water Shortage	0.00	0	0.00	0	0.78	2	0.81	1
Health Issues	0.59	2	0.72	1	0.84	1	0.39	4
Poor Communication Infrastructure	0.58	3	0.72	1	0.56	4	0.48	2
Insecurity & Illegal activities	0.88	1	0.69	2	0.29	11	0.20	10
Unsafe Water	0.48	6	0.55	5	0.36	8	0.00	0
Market Constraints	0.57	4	0.62	3	0.34	9	0.25	7
Poor Education	0.09	13	0.55	5	0.51	5	0.42	3
Poor Leadership	0.24	10	0.60	4	0.00	0	0.30	6
School Shortage	0.00	0	0.50	7	0.22	13	0.00	0
Insufficient Farming Knowledge	0.54	5	0.19	10	0.43	7	0.25	7
Unemployment	0.14	12	0.27	9	0.61	3	0.00	0
Land Shortage	0.00	0	0.52	6	0.22	13	0.24	8
Soil Infertility & Erosion	0.45	7	0.00	0	0.20	14	0.00	0
Environmental Degradation	0.26	9	0.30	8	0.23	12	0.00	0
Financial Resources	0.32	8	0.00	0	0.06	16	0.34	5
Food Shortage	0.00	0	0.07	11	0.44	6	0.15	11
Weather Conditions	0.19	11	0.00	0	0.30	10	0.12	12
Energy Resources & Supply	0.19	11	0.00	0	0.16	15	0.05	13
Household Disputes	0.00	0	0.04	12	0.03	17	0.21	9

Rj values: 1.00 = high risk incidence & severity, 0.00= low risk incidence & severity

cluded sales of produce to intermediary buyers and the impact of national and international economic conditions on produce prices. Poor education was also considered a problem in all of the sites because of the quality and quantity of the education opportunities available to the participants. Despite recent free primary education initiatives, the expenses associated with educational enrollment (i.e., uniforms, books, supplies, transportation) limited participation in this opportunity. People identified poor education, lack of technology development for site-specific agricultural needs and poor agriculture information dissemination as impediments to farming knowledge.

The sample risk index  $R_j$  mean values and their subsequent ranking for each village/location for problems that were present in at least two sites are listed in Table 2. The top ranked problem and community strategy for each location are described according to information collected during community meetings. However, risk index values were based on an integration of both community-level risks and individual-level rankings. It is important to note that risk index values and ranks did not correlate across the locations. For example, both Sengwere and Meibeki identified health issues as the top ranked risk; however, their index values ranged from 0.72 to 0.84. This means that while health issues were similarly important, more residents in Meibeki ranked health issues as a concern than Sengwere, as they both felt the issue was a problem for 100% of the population.

**Regional Risk Trends**

Figure 1 shows the categories of village risks where at least one village had an index value greater than or equal to 0.50 on a scale where 1.00 is the most severe. Using a risk index value of 0.50 as the cutoff helps to illustrate trends between sites of incidence and severity. Sengwere had the highest number of highly ranked risks ( $n=9$ ) that were considered to affect a larger percentage of the population between the four study sites. The higher number of identified risks above 0.50 in Sengwere may be a result of recent resettlement in Sengwere, resulting in lack of infrastructure that may reduce risks. The Moiben region study sites, Meibeki ( $n=5$ ) and Barsombe ( $n=1$ ), had fewer risks that affected a larger proportion of the population or risks that did not have high rankings. Munyaka and Sengwere in the Kapolet region represented areas with a greater number of risks with high severity and incidence rates. The Moiben region sites, Meibeki and Barsombe, collectively had a greater number of identified risks, though not as severe. Risks with statistically significant different mean values between the regions are reported in Table 3, a value of 1 indicates Kapolet had a greater mean index value and a value of 2 indicates Moiben had a greater mean index value as generated from independent samples' Mann-Whitney test and evaluated at  $\alpha \leq 0.05$ . Risks that were not significantly different among the regions included health issues, insufficient farming knowledge, unemployment, land shortage, limited energy resources, and inadequate financial resources.

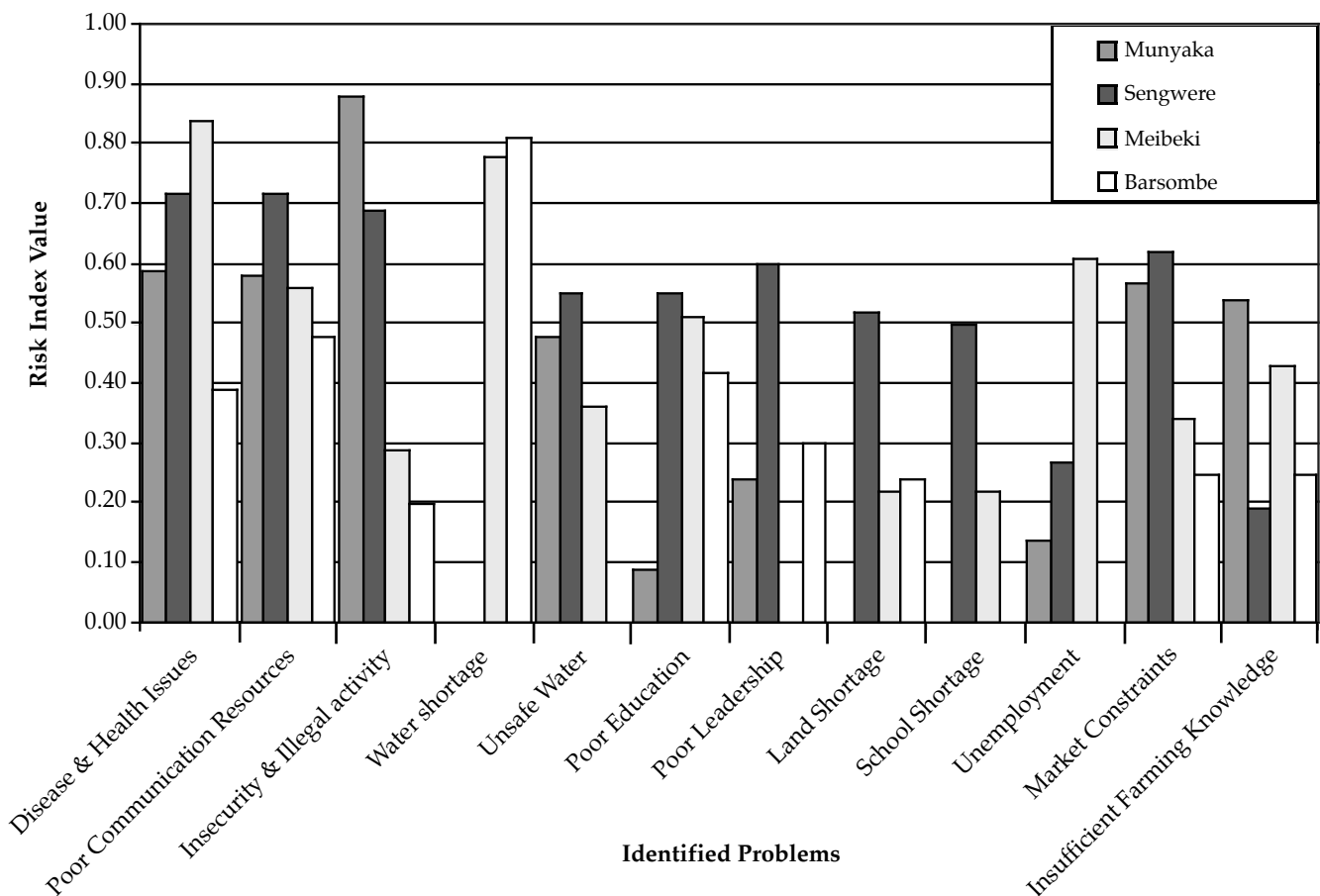


Figure 1. Risks Index Values  $\geq 0.50$

Table 3. Significant Risks  $p < 0.05$

Identified Risks/Problems	Region	Sex	MK	SG	MB	BS
Water Shortage	2				M	
Health Issues						
Poor Communication Infrastructure	1	F				F
Insecurity & Illegal activities	1	F			F	F
Unsafe Water	1	F			F	
Market Constraints	1	M			M	M
Poor Education	2			F	F	M
Poor Leadership	1		F	M		M
School Shortage	1	F			F	
Insufficient Farming Knowledge		M		M		M
Unemployment			F	M		
Land Shortage		F		F	M	F
Soil Infertility & Erosion	1	M	M		M	
Environmental Degradation (deforestation)			F	F	M	
Financial Resources					M	F
Food Shortage	2			M	M	F
Weather Conditions (droughts, floods)	2		F			M
Energy Resources & Supply	1	M	M	M	M	M
Household Disputes	2	F		F	F	F

MK=Munyaka, SG= Sengwere, MB=Meibeki, BS=Barsombe

1 = Kapolet region (Munyaka and Sengwere villages)

2 = Moiben region (Meibeki and Barsombe locations)

M = Men, F = Women

### Gendered Risks

Men’s and women’s meetings were held in each location to identify specific risks and assets of importance. Risks evaluated on a gender basis allowed risks to be weighted appropriately, as men and women had different problems or felt risks were more or less severe to them and their community. Individual risk index values ( $n=10$  per village) were calculated to allow for independent samples Mann-Whitney test analyses to compare risk index means by gender.

Table 3 lists the risks where M indicates a statistically higher mean risk index value for men and F indicates a statistically higher mean risk index value for women. Risks were uniquely generated in each meeting allowing some risks to be identified by only one gender. Several risks developed as being gender related problems as they had higher significant mean risk index values across all the study sites. Energy resources and supply was a risk only identified by men causing the statistical significance between genders. The concern about energy sources and supply may be a result of decline in forest resources and access to them. In the Kapolet region, the Kapolet forest

reserve remained as an important source for charcoal production, which was an income generating albeit illegal activity for men. Charcoal production was observed during transect walks and household interviews. In the Moiben region, energy source concerns were more focused on fuel for tractors and electricity.

Household disputes were only a concern with women as shown in three of the four study sites. Household disputes encompassed domestic violence, confrontation with neighbors, illegal home breweries, and were associated with lack of employment opportunities. Women were often involved in home brewing of alcohol because they felt it was an important income generating activity. However, men were the main consumers of this alcohol and this often resulted in a reduction of their homestead financial resources and contributed to domestic violence. Agricultural production was predominately a concern with men, as reflected in the men having a statistically higher mean index values for market constraints, insufficient farming knowledge, and concerns with soil fertility and erosion. Many of these concerns were specifically focused on the production and marketing of maize as described in the community meetings. Men were typically responsible for securing financial resources to purchase farm inputs (e.g., certified seeds, fertilizer, labor, and tractor hire).

## Assets

Livelihood vulnerability consists of risks and assets. These assets are not only natural resource assets and basic needs but also consist of human, social, economic, and cultural capital (Bebbington, 1999). Bebbington (1999) described assets as capital that gives meaning to a person's life and gives them the capability to improve their quality of life. An attempt was made to generate a list of assets during the meetings with each gender, but this list did not go beyond basic needs. During the community meeting setting, it was difficult to get participants to talk about what comprised community strength, what resources were unique or particularly useful, and their strategies used for eliminating risks. Specific assets for each community were summarized from key informant interviews, personal observations, and household questionnaires.

Notable assets in Munyaka included church groups, merry-go-round groups, and extension services. An active Ministry of Agriculture extension officer, a Swedish nongovernmental organization Vi Agroforestry extension person, church associations, and community-based organizations were actively developing workshops, securing funding, finding sponsors, and providing trainings in Munyaka. The use of merry-go-round groups was suggested by key informant interviews and household respondents as an important local method of saving money and securing labor. Merry-go-rounds functioned as a group of people who pool a collected resource to give to one participating individual. This collection and distribution is cycled through all the participants until all individuals have been compensated for their original investment. However, a few people suggested they did not trust other community members enough to participate in merry-go-rounds.

Assets in Sengwere included fertile soils, community revitalization activities, and services provided by a Ministry of Agriculture extension officer. A unique asset found in Sengwere was the establishment of a cultural center that promoted preservation of tribal customs and provided education opportunities. In Sengwere, an active and knowledgeable Ministry of Agriculture officer provided community education classes ranging in topics from HIV/AIDS awareness, beekeeping, and soil and water conservation. The Ministry of Agriculture officer felt that the people of Sengwere were open and receptive to instruction on new methods to manage their resources that would potentially improve their livelihoods. Household respondents indicated that they did not participate in merry-go-round groups because they were new to the area and did not know their neighbors well enough or that they did not have capital for the original investment.

The people in Meibeki felt they lived in a welcoming and peaceful community. Crime was low because of local citizens that were vigilant and police monitoring. The assistant chief was active and held weekly court sessions that held people accountable for wrongdoings. In addition, during data collection, the assistant chief held several meetings to assist with voter registration for the upcoming constitution referendum vote. Women's groups helped mobilize funds for its members and a youth group was participating in reforestation activities. The aid organization World Vision had funded education and general health sensitization.

One notable asset in Barsombe was the formation and registration of a women's group as a local community-based organiza-

tion, which was eligible to apply for funding of local activities. Other women's groups, youth groups, and church activities were all indicated in household questionnaires as resources for community members. Out of the four study sites, Barsombe had the best access to markets on a tarmac road.

## DISCUSSION

The risks identified during community meetings by all study sites included: disease and health issues, insecurity and illegal activities, poor communication resources, insufficient markets, limited farming knowledge, and poor education (Table 2). Risks that did not have a significant mean risk index difference among the regions (health issues, insufficient farming knowledge, unemployment, land shortage, environmental degradation, and limited financial resources) may indicate that these are problems occurring on a larger scale and are a result of changing resource use, resource user, and political conditions nation-wide (Thomas-Slyter and Rocheleau, 1994). One risk of growing concern to agricultural communities is HIV/AIDS. HIV/AIDS infection is highest in people between ages 15–45, who are the most productive work force members (RoKMoFP, 2001). The World Health Organization (2004) reported a 6.7% HIV/AIDS infection rate in Kenya with over 30% HIV/AIDS infection in some urban areas, including western Kenya. Although HIV/AIDS did not emerge as an explicit risk in the study sites (note: HIV/AIDS is a sensitive subject not frequently discussed because of the associated negative stigma), participants were concerned about their vulnerability to disease. However, poor infrastructure and remoteness of study site communities limits peoples' movement to urban areas with higher infection rates and may actually help slow transmission of the virus to rural communities.

The risk index method developed by Smith et al. (2000) showed heterogeneity within and between their research sites in Northern Kenya and Southern Ethiopia and noted differences between gender and wealth status. Risks indicated by participants in the Kapolet and Moiben region community meetings (human disease, insecurity, water shortage, food shortage, land shortage education limitations, and market constraints) were similar to those found by Smith et al. (2000). Heterogeneity occurred between the Kapolet and Moiben regions and between genders as unique risks and assets created varying vulnerability levels (Table 3). Gendered risks reflected men's concerns with agricultural production and women's concerns reflected household well-being and sustainability. A similar trend was also found within pastoral communities studied by Smith et al. (2000).

### Community Capacity

How a community responds to risk is influenced by the socioeconomic and biophysical context, the perceived severity of the risk among community members and the local collaborative capacity of the community members to solve problems (Flint and Luloff, 2005). Communities with a high degree of collaboration are more likely to act on perceived risks whereas communities with low levels of interactivity are less likely to respond to risks, even if those risks are severe and widespread (Flint and Luloff, 2005). The community where one lives influences individuals' livelihood strategies with impacts on resource availability, educational sources, social networks and governmental representation. Activities in Munyaka suggest the potential to increase commu-

nity collaboration for addressing individual and community-wide risks. Insecurity was the top ranked risk in Munyaka and was being addressed through the utilization of available police enforcement. Due to the physical and socioeconomic context of cattle theft (i.e., cattle thieves were from a different tribe and different geographic locations, and loss of cattle resulted in loss of wealth), people in Munyaka were able to view cattle theft as a community-wide threat. Activities demonstrated by both men's and women's groups, merry-go-round groups, and active extension personnel have set positive examples for further collaboration that may assist in reducing the severity of other risks. The low number of gender-differentiated risks indicates that the majority of risks identified may be viewed as community-wide and non-gendered discriminatory risks.

High-risk severity and incidence in Sengwere can be attributed to the resettlement process of forming a new community. Their limited access to resources is also a consequence of resettlement. Most resettled families were given 2.5-acre plots on steep slopes. Even though the land was considered very fertile, most families struggle to meet their subsistence needs. However, the community's ability to try to strengthen broken tribal customs with the formation of a community cultural center, identification of the need for a strong community leader and high attendance at sensitization activities provide strong support for the notion that over time more collaborative activities may develop to assist in reducing risks.

In both Meibeki and Barsombe, water shortage was a major community-wide risk. Water shortage stimulates other identified risks such as health issues, food shortages and crop production limitations. Water shortage in the Moiben region is attributed to unpredictable weather conditions that can severely alter the water quality and quantity. Livelihood conditions are dependent on water supply as time and energy is spent on water collection. Water management is often an important unifying factor in a community (Cook et al., 2003). However, this research did not find a strong community mobilization focused on resolving their water issues. Some of the notable assets that assisted with the water shortage were donkeys and tractors; those people without transport often bought water. Water is a commodity that could justify why community mobilization had not centered on resolving the water shortage. Individual strategies reflected coping, helplessness and a dependency on external assistance to deal with the water shortage problem. With the assistance of a local aid agency establishment of a piped water system and storage tanks were being discussed. However, for individual households to participate a water pipe fee is required. This potentially continues the cycle of those people who are the most vulnerable to water shortage (lack of transport resources) to remain vulnerable because they likely lack sufficient financial capital for pipe fee investment.

### CONCLUSIONS

The risks and assets identified in the Kapolet and Moiben regions most likely represent a small number of the actual risks and assets in each community, as individual vulnerability is based on how they manage their risks and assets to create resilience and reduce exposure. The risks that individuals and the community have not successfully addressed are likely the top ranked risks in each community. This could also justify why generating a list of assets was so difficult for community participants, as capital resources may seem important or unimportant depending on their current actual and perceived risks.

Unique strategies are likely to have already alleviated past risks by drawing upon the assets that were available.

Few (2003:52) states, "it is increasingly accepted that people do not simply draw on their assets but possess sophisticated skills in managing them to cope with adversity and take advantage of opportunities." People in the Kapolet and Moiben region do take advantage of their assets in a manner that mitigates risks. On an individual level, management of specific assets contributes to increasing other assets, such as human, financial and social capital. Examples included farmers in the Kapolet region using available financial assets to diversify crop production to avoid risk of crop failure and market constraints, and women in the Moiben region committing time and labor to women's gardening groups to reduce risk of food insecurity and strengthen social networks. In the Kapolet region, on a community level, social assets are strengthening with the assistance of MoA extension and Vi Agroforestry services that encourage the formation of and participation in community groups to further their educational opportunities and improve their natural resource base. Within the Kapolet region, the growing social networks allowed communities to overcome some resource deficiencies (poor communication and governmental representation) to seek assistance with their insecurity risks. Prior community group experiences may have enabled the community to adjust and activate existing social networks to increase community awareness and vigilance to the risk of cattle theft. In the Moiben region, community response to their largest risk, water shortage, was dependent on their association with aid agencies, governmental representatives and their subsequent ties with influential people.

The evolving process of building resilience, mitigating risks and accumulating assets makes identifying individual and community vulnerabilities difficult to manage. Improving current deficiencies in physical assets, such as communication infrastructure would begin to mitigate risks by improving access to health facilities, markets, credit institutions and government offices that are all limited in the study locations by distance. With increased access to these resources, people may employ new livelihood strategies to build resilience and accumulate assets to help alleviate risks. However, improving physical assets is highly dependent on political policies and the current political interest in the area. This then suggests that local management policies need to target risks that individuals and the community have successfully mitigated or build on assets that are attainable within the community.

### ACKNOWLEDGMENTS

We would like to acknowledge Dr. Mucai Muchiri of Moi University, Vi Agroforestry personnel in Kitale, and the Ministry of Agriculture extension officers for their guidance and assistance. I also want to thank Jacob Cheruiyot Ngetich, Scholastica Wekhuyi and Wycliffe Wanambisi Wesakania for their dedication to the project, enduring long field days, and maintaining a sense of humor through the whole process. Most importantly, we would like to acknowledge the people of Munyaka, Sengwere, Meibeki, and Barsombe for participating and welcoming the research team into their communities. We would also like to acknowledge Heather Patt's graduate committee members at Michigan State University, Tracy Dobson, JD, and Dr. Jennifer Olson for their guidance. Heather would like to thank her friends and family who shared in her excitement of being able to return to Kenya to work on a watershed project.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## FARMERS TRAINING IN TANZANIA

*Twelfth Work Plan, Economic/Risk Assessment and Social Analysis 3 (12ERA3)  
Abstract*

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### ABSTRACT

The Tanzania Fisheries and Aquaculture Development Division depends on farmer training workshops as a major means of sustainable technology transfer in addition to extension services. Most on-farm research activities are conducted by the Sokoine University of Agriculture in collaboration with Kingorwila National Fish Center, Fisheries and Aquaculture Development Division. Both institutions are in the Morogoro Region. The proposed training will involve 25 fish farmers from different participating villages in the Morogoro Region. The training is important for developing model fish farmers who will participate in future research activities and extend the knowledge to other fish farmers in the region.

The training will be a five-day workshop that will be conducted in collaboration with Mkindo Farmers Training Center. The major topics to be covered are pond construction, pond management, fish health, fish nutrition, economics of production, and marketing. Training instructors will come from Sokoine University of Agriculture in Tanzania, Kingorwila National Fish Center, Moi University in Kenya, and University of Arkansas at Pine Bluff, US. The workshop is scheduled to take place in November 2006. The training activities will be based on training modules and trainees include women and household members who manage fish ponds.

It is anticipated that farmers will acquire knowledge that will be used to improve farm productivity. The training will help to accelerate the adoption process of improved technical innovations (through farmer-to-farmer knowledge transfer).





# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## EX ANTE ASSESSMENT OF COASTAL AND MARINE AQUACULTURE DEVELOPMENT: CHARTING THE STRENGTHS AND WEAKNESSES OF LOW TROPHIC SPECIES FOR OFFSHORE AQUACULTURE IN DEVELOPED AND DEVELOPING COUNTRIES

*Twelfth Work Plan, Economic/Risk Assessment and Social Analysis 5 (12ERA5)  
Abstract*

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### ABSTRACT

This report examines the ex ante development of low trophic marine organisms in exposed ocean conditions with an emphasis on the developing world. Overall, we found an overwhelming preference for high-value finfish culture regardless of location; high value product being deemed necessary to offset the large costs and risks associated with farming in exposed ocean sites. This focus has tended to obscure attention on the primary utilization of low trophic marine species in the development of exposed ocean culture systems. Drawing from a series of case studies, interviews and literature review, we first provide a series of sustainable developmental criteria that must be met; site selection, biological and economic factors related to culture systems, property rights, environmental standards and contributing to community development and avoiding user conflicts need much more consideration. We then examine ten low trophic candidate species in terms of their sustainable development potential. Our findings reveal that at present, sponge, blue mussel and perhaps pearl culture may warrant some further examination. For developing countries, offshore aquaculture of low trophic species must compete with near shore systems that hold marked advantages in terms of economic and social economies of scale. In exposed ocean environments, high investment costs, established technology, managerial expertise and achieving efficient economies of scale in both production and post-harvest phases will remain significant obstacles for future sustainable development efforts in developing



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## BIVALVE MARKET STUDY IN PACIFIC MEXICO

*Twelfth Work Plan, Economic/Risk Assessment and Social Analysis 6 (12ERA6)*  
Abstract

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### ABSTRACT

This work proposes to build on past and current efforts on the Pacific Mexico coast designed to promote viable aquaculture alternatives other than shrimp mariculture with the goal of optimizing returns and benefits to rural, coastal communities. Previous work conducted by a multi-institutional, international team since 1997 has built a solid foundation for diversification of aquaculture in Pacific Mexico, emphasizing the use of native species, particularly those low on the food chain and with low culture technology requirements. Among the leading candidates are bivalves, which are currently cultured and harvested extensively along the Gulf of California Coast, with most production occurring as a result of fisheries. Great potential exists to expand current aquaculture production through strengthening existing operations and developing new species of bivalves for culture.

This work will be conducted in the context of a long-standing, multi-institutional effort to develop and implement management plans for two important and extensive wetlands areas on the Pacific Coast of Mexico: Bahía Santa María (BSM), Sinaloa State, and Boca Camichin, Nayarit State. The latter is the site of a thriving, small-scale oyster industry based on the native Mexican oyster, *Crassostrea cortezensis*. Although BSM has some oyster culture, stakeholder interest is strong in developing more oyster culture and developing other species of valuable bivalves. During the past two years of collaborative work, bivalves (clams, oysters, pen shells, etc.) were identified as the culture candidates with the most potential to offer an alternative to shrimp farming for coastal communities of Mexico. Shellfish culture also offers more opportunities for participation of women and other marginalized groups due to their ease of culture and low-input requirements. Bivalves, however, are particularly demanding in the area of sanitation and food quality because they are filter feeders and are sensitive to post-harvest contamination. Resolving water quality, handling, processing, marketing, and transportation issues related to production of safe bivalves will not only help improve the benefits and reduce risks associated with this form of culture, but will also serve as a model for improved sanitation for other species such as finfish, which are targeted for development.

The Pacific Aquaculture Center/University of Hawaii at Hilo (PACRC/UHH), the Universidad Autónoma de Sinaloa (UAS), Autonomous University of Nayarit (UAN), Research Center for Food and Development (CIAD), Coastal Resources Center/University of Rhode Island (CRC/URI), Regional Center for Education for Sustainable Development (CREDES), Sinaloa Aquaculture Institute (ISA), Sinaloa Committee for Aquaculture Sanitation (CESASIN), community groups, and industry cooperators have been working together under ACRSP funding for two years. The University of Alaska/Sea Grant and the University of Louisiana Sea Grant College Program have also been recently integrated into the regional Latin America initiatives of the collaborators. Economists from CIAD (Francisco Cordero Martinez) and the University of Alaska (Quentin Fong) will take the lead on this work.

There is little information on marketing channels, opportunities, prices, and consumer preferences for bivalves. There is also a lack of clarity as to the regulatory nature of this field and the requirements for implementation of existing regulations. While the coastal communities involved in this work in Sinaloa and Nayarit are surrounded by major cities (e.g., Culiacan), tourist destinations (Acapulco, Mazatlan), and are close enough to the US to export bivalves, more information is needed on the economics and markets to inform current and future efforts.

Important research questions to be answered are: What prices is the market willing to pay for the BSM bivalves? What is the volume or quantity demanded? What are the current federal and state regulations concerning shipping of bivalves and environmental considerations for culture systems? Are regulations that prohibit transportation of live food due to disease transmission concerns? What regulatory measures are needed to assure safe shipping of bivalves? What is the supply outlook for bivalves in the main local, regional, and national markets? And finally, can BSM's and Nayarit's producers be suppliers of high quality bivalves in the domestic or international markets? If not, what are the constraints to growth? This work intends to address these questions.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## INTEGRATED CAGE-CUM-POND CULTURE SYSTEMS WITH HIGH-VALUED CLIMBING PERCH (*ANABAS TESTUDINEUS*) IN CAGES SUSPENDED IN CARP POLYCULTURE PONDS: BANGLADESH

*Twelfth Work Plan, Applied Technology and Extension Methodologies 1a (12ATE1a)*  
Abstract

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### ABSTRACT

An on-farm trial was conducted to evaluate the growth performance of caged climbing perch (*Anabas testudineus*) with six carp species (*Catla catla*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Cirrhinus cirrhosus*, *Barbodes gonionotus*, and *Cyprinus carpio*) stocked in the open water of 18 rural ponds for 150 days in the Mymensingh region of Bangladesh. One 1-m<sup>3</sup> cage per 200 m<sup>2</sup> pond area was suspended in each of 12 earthen ponds and the remaining six ponds served as controls without cages. Climbing perch fingerlings of 2–3 g in size were stocked at 200 and 400 fish m<sup>3</sup> in cages, while carp fingerlings of 8–15 g size were stocked at 1 fish m<sup>2</sup> in all 18 ponds, giving caged climbing perch to open-pond carp ratios of 1:1 and 2:1, respectively. Caged climbing perch were fed on Saudi Bangla commercial pelleted feed (32.38% crude protein) for the first 90 days and grower feed (38.06% crude protein) for the rest. Feeds were supplied at 100% body weight per day for the first month and at 5% body weight per day for the rest of the culture period. No additional supplemental feeds were supplied for carp production in control or treatment ponds.

Survival of climbing perch was 40.4% in the 1:1 treatment and 29.5% in the 2:1 treatment; these values were not significantly different ( $P>0.05$ ). There was no significant ( $P>0.05$ ) difference in survival of carp either, ranging from 41% to 93%. Final mean weights of both climbing perch and carp were not significantly different ( $P>0.05$ ) among all treatments, ranging from 15.1 to 20.1 g and from 235.3 to 341.1 g, respectively. Total harvest weight of climbing perch was not significantly different ( $P>0.05$ ) between treatments (3.04 ± 1.28 and 2.49 ± 2.24 kg per pond in the 1:1 and 2:1 ratio treatments, respectively). Production performance of carp was not significantly different among treatments. The combined total weights of both climbing perch and carp were not significantly different between treatments. Net revenues were positive but comparatively low in all treatments. The results indicated that the caged climbing perch to open-pond carp ratio 1:1 was better, but stocking size of climbing perch fry should be larger. More on-farm trials in different ecosystems are necessary to develop the technology for further dissemination among the rural farmers.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## INTEGRATED CAGE-CUM-POND CULTURE SYSTEMS WITH HIGH-VALUED AFRICAN CATFISH (*CLARIAS GARIEPINUS*) IN CAGES SUSPENDED IN CARP POLYCULTURE PONDS: NEPAL

*Twelfth Work Plan, Applied Technology and Extension Methodologies 1b (12ATE1b)*  
Abstract

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### ABSTRACT

This on-farm trial was carried out for 164 days in 18 earthen ponds of 85–130 m<sup>2</sup> in surface area in three sites of Nepal to adapt integrated cage-cum-pond systems to local conditions in Nepal and to verify best results of the on-station trial. One cage (1.5 x 1.5 x 1.0 m), with water volume of 2 m<sup>3</sup> and covered with 1-cm mesh net, was suspended in each of the treatment ponds. There were two treatments: 1) carp at 1 fish m<sup>-2</sup> in open ponds without cages (control); and 2) African catfish (*Clarias gariepinus*) at 100 fish m<sup>-3</sup> in cages and carp at 1 fish m<sup>-2</sup> in open ponds (caged treatment). Each trial site had three replicates for both the control and treatment. African catfish fingerlings of 12.8–13.2 g in size were stocked in cages, while fingerlings of silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigala*), with average weights of 4.6, 2.2, 4.2, 0.5, and 0.7 g, respectively, were stocked in the open water of all ponds. The stocking ratio of silver carp, bighead carp, common carp, rohu, and mrigal was 4:2:2:1:1, respectively, in each pond. Caged catfish were fed twice daily with a locally made pellet feed (28% crude protein), while no feed or fertilizer was added into open water. In the control, ponds were fertilized weekly with diammonium phosphate (DAP) and urea at rates of 2 kg N and 1 kg P ha<sup>-1</sup> d<sup>-1</sup> throughout the experimental period.

Survival of African catfish, ranging from 53.3% to 56.8%, was not significantly different among all sites. Daily weight gains (1.2 to 1.4 g fish<sup>-1</sup> d<sup>-1</sup>), gross fish yield (19.0 to 25.4 kg cage<sup>-1</sup> crop<sup>-1</sup>), net fish yield (16.6 to 22.9 kg cage<sup>-1</sup> crop<sup>-1</sup>), and feed conversion ratio (2.6 to 3.3) of African catfish were not significantly different among all sites. Most growth and production parameters of carps were not significantly different between the control and treatment ( $P > 0.05$ ). Both the caged treatment and control ponds produced positive net returns (NRs) with 1860 NRs/100-m<sup>2</sup> pond in the caged treatment ponds, and 1400 NRs/100-m<sup>2</sup> pond in the control ponds in one culture cycle.

African catfish has the potential to be cultured in an integrated cage-cum-pond culture system, but it is necessary to fine-tune stocking ratios of catfish to carp and escape the winter season for culture.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## INTEGRATED CAGE-CUM-POND CULTURE SYSTEMS WITH HIGH-VALUED CLIMBING PERCH (*ANABAS TESTUDINEUS*) IN CAGES SUSPENDED IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) PONDS: VIETNAM

*Twelfth Work Plan, Applied Technology and Extension Methodologies 1c (12ATE1c)*  
Abstract

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### ABSTRACT

This on-farm trial was carried out in three districts of Vietnam (Tam Binh district of Vinh Long province, Thot Not district of Can Tho city, and Vi Thuy district of Hau Giang province) to adapt integrated cage-cum-pond systems to local conditions. Five earthen ponds of 100 m<sup>2</sup> in surface area were selected in each of the three sites for the on-farm trial.

Nile tilapia (*Oreochromis niloticus*) fingerlings (8–10 g size) were stocked at two fish m<sup>-2</sup> in all ponds, while climbing perch (*Anabas testudineus*) fingerlings (8–10 g size) were stocked in a 4-m<sup>3</sup> cage suspended in each treatment pond. Stocking density of climbing perch was the treatment variable and was 50, 100, 150, and 200 fish m<sup>-3</sup>, giving caged climbing perch to open-pond Nile tilapia ratios of 1:1, 2:1, 3:1, and 4:1. There were also control ponds without a cage (0:1), which were fertilized weekly with urea and diammonium phosphate (DAP) at 28 kg N and 7 kg P ha<sup>-1</sup> week<sup>-1</sup>. No fertilizer was added into treatment ponds. Pelleted feeds containing 32%, 26–28%, and 22% crude protein were given twice daily to caged climbing perch during the first, second and remaining months at rates of 5%, 3%, and 2% body weight per day, respectively.

Survival of climbing perch, ranging from 85.5% to 91.1%, was not significantly different among sites and treatments. Daily weight gain (0.28 g fish<sup>-1</sup> day<sup>-1</sup>) of climbing perch was significantly higher in the 1:1 ratio treatment than those (0.16–0.17 g fish<sup>-1</sup> day<sup>-1</sup>) in other treatments ( $P < 0.05$ ), among which there were no significant differences ( $P > 0.05$ ). Total harvested climbing perch biomass, ranging from 8.77 to 23.7 kg cage<sup>-1</sup>, increased with increasing stocking ratio of climbing perch to Nile tilapia ( $P < 0.05$ ). Feed conversion ratio (FCR) was lowest in the 4:1 ratio treatment, intermediate in the 1:1 and 3:1 ratio treatments and highest in the 2:1 ratio treatment ( $P < 0.05$ ). Survival of Nile tilapia was highest (93%) in the 3:1 ratio treatment, intermediate (86.8%–89.3%) in the 0:1, 1:1, and 2:1 ratio treatments, and lowest (84%) in the 4:1 ratio treatment ( $P < 0.05$ ). Growth of Nile tilapia, ranging from 1.17 to 1.78 g fish<sup>-1</sup> day<sup>-1</sup>, was not significantly different among treatments ( $P > 0.05$ ), while total harvested tilapia biomass was highest in the 3:1 ratio treatment, intermediate in the 1:1, 2:1, and 4:1 treatments, and lowest in the 0:1 ratio treatment (control) ( $P < 0.05$ ). Treatments with higher ratios (3:1 and 4:1) gave higher net revenues (0.374 and 0.361 million VND per 100 m<sup>2</sup> pond).

The on-farm trial has demonstrated that the high-valued climbing perch may provide potential for the integrated cage-cum-pond culture system, but it is necessary to improve FCR of climbing perch in order to increase the profitability of the system.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## AQUACULTURE TRAINING FOR KENYAN EXTENSION WORKERS, FISH FARMERS, AND UNIVERSITY STUDENTS

*Twelfth Work Plan, Applied Technology and Extension Methodologies 3 (12ATE3)*  
Abstract

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### ABSTRACT

The ACRSP, the Moi University (MU) Department of Fisheries and Aquatic Sciences, and the Fisheries Department of the Government of Kenya have expended considerable effort on aquaculture training at various levels during the past decade. Target audiences for this training have included fish farmers, fisheries extension workers, undergraduate students, and graduate students. Training for fish farmers and extension workers has typically been conducted through farmer field days and two- and three-week short courses. Training for undergraduates typically has involved providing small stipends and supervision for "senior projects" in some aspect of aquaculture appropriate to Kenya. Training for graduate students has been done by providing scholarship support for formal degree programs, both abroad and at Kenyan Universities.

This investigation was undertaken to continue these training efforts in Kenya. Specific objectives have been to train up to 34 extension workers and six advanced farmers in hatchery management techniques, to provide on-farm training in simple techniques for spawning, hatching, and rearing catfish juveniles in ponds for up to 12 farmers, to provide stipend support for four undergraduate students training in aquaculture at MU, and to provide scholarship support for two Master's-level (M.Sc.) university students at MU.

All objectives of this investigation have been met, with the exception that the two M.Sc. students have not finalized their theses for submission to the MU Graduate School. Two two-week short courses were given to selected FD Fisheries Assistants (extension workers), KMFRI research officers, and advanced fish farmers. The courses were held at Sagana and Moi University, from 16–31 April and 15–28 August respectively. Twenty individuals were trained in each session. The courses focused on the African catfish (*Clarias gariepinus*) fingerling production process, from maintenance of broodstock through brooder selection, spawning, incubation, hatching, and rearing of fry to the fingerling stage. In addition, two on-farm training sessions were conducted for advanced farmers during 2005 and early 2006. The first fish farmers training was held at both Chepkoilel campus and Kesses next to Moi University main Campus from 19–21 May 2005. The second training was held at Chepkoilel Campus from 2–5 April 2006. The training consisted of hands-on spawning/hatching/rearing work conducted by the farmers themselves under the guidance of a host farmer and one or more experienced technicians from MU and the FD. Four MU undergraduate students received support for their senior project work and two graduate students received full scholarship support. The graduate students have completed their field work and data analysis and are currently in the final stages of preparing their thesis for submission. It is expected that they will graduate by December 2006.

Completion of this investigation will benefit Kenya and the region in many ways. Extension workers and fish farmers will be able to apply new knowledge to increase *Clarias* fingerling production on government and private farms. An increased supply of *Clarias* fingerlings will provide Lake Victoria Nile perch fishers with a reliable source of bait and fishing pressure on immature *Clarias* in the lake will decrease. A steady supply of *Clarias* fingerlings will also help producers in areas where *Clarias* is gaining popularity as a cultured food fish, and farmers producing *Clarias* fingerlings will enjoy an additional source of income. Increases in fish production realized through all these avenues will contribute to human health and welfare in the region.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## TRAINING LOCAL FARMERS ON SAFE HANDLING OF STEROIDS AND MASCULINIZATION TECHNIQUES IN CENTRAL AMERICA

*Twelfth Work Plan, Applied Technology and Extension Methodologies 4 (12ATE4)  
Final Report*

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### ABSTRACT

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 from farmers and identify problems that may compromise advances in the field of interest. 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 implemented three workshops in Central America on safe handling of steroids and masculinization techniques.

### INTRODUCTION

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 widespread 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 developed regional workshops on safe handling of steroids and masculinization techniques in Guatemala, Honduras and Costa Rica.

#### METHODS AND MATERIALS

Training activities were offered as extensive workshops to farmers, researchers, extension agents, and students. Two major topics were covered: fish sex inversion and safe handling of steroids. Printed and electronic materials for safe handling of steroids and masculinization techniques were distributed during training activities. Carl Schreck, Grant Feist, Guillermo Giannico, Ulises Hernández, Alejandro Mcdonal, Bernardita Campos and Wilfrido Contreras constructed PowerPoint presentations that were used in workshops. These presentations included information on fish sex inversion, safe handling of steroids, diagrams of filtration systems and videos.

Table 1. First Regional Workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. San Pedro Sula, Honduras, Extension agents, students and small farmers. UJAT-OSU. This workshop took place at Hotel Gran Sula facilities. October 13, 2005.

	Name	Institution	Gender	Nationality
1.	Jairo E. Amézquita G	Planta ALCON-Alim.	Male	Honduran
2.	Franklin A. Vélchez Molina	Researcher	Male	Nicaraguan
3.	José D. Meza Aguilar	Researcher	Male	Nicaraguan
4.	Tomas Arita Valle	Rural farmer	Male	Honduran
5.	Carlos J. Arita Paz	Rural farmer	Male	Honduran
6.	Luis E. Valle Coello	Rural farmer	Male	Honduran
7.	José W. Lanza Nuñez	Rural farmer	Male	Honduran
8.	José D. Rodríguez	Comercial la Rosa	Male	Honduran
9.	Alex O. Martínez Sauzo	Extension agent	Male	Honduran
10.	Saulo G. Ávila Sauzo	Extension agent	Male	Honduran
11.	Constantino Alvarado Hdz.	Consultant	Male	Honduran
12.	Luis A. Varela Suaréz	Consultant	Male	Honduran
13.	Nelson E. Martínez Sandoval	Farmer	Male	Honduran
14.	Héctor R. Castillo Melgar	Rural farmer	Male	Honduran
15.	Mario I. Rodríguez Tercero	Farmer	Male	Honduran
16.	Héctor E. Sauzo Chinchilla	Planta ALCON- Alim.	Male	Honduran
17.	Cornelio Ramos Martínez	Extension agent	Male	Honduran
18.	Federico Mazariegos	Extension agent	Male	Honduran
19.	Erasmus Pinto Cansinos	Rural farmer	Male	Honduran
20.	Carla Lizzeth Garces	Student-Zamorano	Female	Honduran
21.	Johy S. Talhami Vázquez	Farmer	Male	Honduran



Workshops were implemented as follows:

First regional workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Hotel Gran Sula, San Pedro Sula, Honduras. October 13, 2005. Support for this workshop in Honduras was provided by Dr. Dan Meyer and MsC Suayapa Meyer, Escuela Agrícola Panamericana Zamorano.

Second regional workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Ciudad de Guatemala, Guatemala. December 01–02, 2005. Support for this workshop in Guatemala was provided by Dr. Leonel Carrillo-Ovale, Centro de Estudios del Mar y Acuicultura (CEMA), Universidad de San Carlos de Guatemala (USAC).

Third regional workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Heredia, Costa Rica. June 22–23, 2006. Escuela de Ciencias Biológicas, Campus Omar Dengo, Universidad Nacional de Costa Rica (UNA). Support for this workshop in Guatemala was provided by Dr. Juan Ulloa, Universidad Nacional de Costa Rica.

## RESULTS

*First regional workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Hotel Gran Sula, San Pedro Sula, Honduras. October 13, 2005.* Due to time constraints, this workshop was conducted in one day and covered only the theory behind sex inversion and safe handling of steroids. There were 21 participants at the workshop. MsC. Ulises Hernández-Vidal gave the morning lecture and discussed producing suitable tilapia broodstock for fry production. The lecture covered basic principles of reproduction in tilapia, how to transport fish, identify sex and spawning conditions. Ulises explained how to select broodstock (based on length and condition factor) to obtain a line of fish that would generate high quality fry. MsC. Alejandro Mcdonal-Vera lectured on fry production, broodstock sex ratios, tilapia reproductive cycles, fry collection, management and selection of fry. The theory for masculinizing techniques was covered.

In the afternoon session Ulises gave a detailed presentation on the methodology for sex reversing tilapia using steroid treated food. He included temperature and duration of treatment as important components in the process. Sorting and grading of fry to select fish at the appropriate stage for sex reversal was documented. The methodology to incorporate steroids into food was presented, as well as safety precautions when using hormones. Dose and timing of hormone administration was shown for several species. The use of steroids incorporated into live food was presented as a way to sex reverse carnivorous fishes. Finally both Ulises and Alejandro covered all the information related to safe handling of steroids, clean technologies in aquaculture and filtration systems used to eliminate steroids. A general overview on steroid hormone characteristics and how steroids can accumulate in water and sediments following sex reversal of fry was provided. They also discussed the effects of residual steroids on other fish and why it is important to eliminate them. Ulises gave a presentation on the safe handling of steroids and how to eliminate them from aquaculture systems. He described the dangers of steroid exposure for both humans and the environment. He then gave a detailed account of the components for a filtration system to remove steroids and nitrogenous waste including how to construct them. Use of solar light or UV sterilizers was discussed as another method to remove steroids from effluent. Finally, safe handling of steroids and methods to clean work areas after their use was presented.

All materials were used to create PowerPoint® presentations. *Second regional workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Ciudad de Guatemala, Guatemala. December 01–02, 2005.* This workshop comprised a one-day theory session and a one-day practicum session. All materials used at the first regional workshop were also used here. A total of seventeen participants attended the workshop. Fourteen were students and one was a researcher from the Centro de Estudios del Mar y Acuicultura. Two participants were extension agents from the Federal Fisheries Department. On day 2, a practicum session was conducted at the “Centro de Capacitación Piscícola Finca Sabana Grande”. Participants were shown how to grade, sort, and count fry to be masculinized. They were also shown how to make hormone laced food and informed of safe handling practices when working with steroids.

*Third regional workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Heredia, Costa Rica. June 22–23, 2006.* For this workshop Ulises Hernández-Vidal and Alejandro Mcdonal organized presentations and were the main speakers. They used all the materials presented at the first regional workshop using PowerPoint® presentations. A total of thirty participants attended the workshop. Ten attendees were researchers from the National University, seven were farmers, six were students; three were extension agents, two were government officials and two were representatives of the feed industries. The practicum session was conducted in the University’s facilities.

## DISCUSSION

Universidad Juárez Autónoma de Tabasco (UJAT) has become a major source for training in Central America. The laboratory of aquaculture has taken the lead on clean technologies for aquaculture and safe handling of steroids. Students, researchers, extension agents, private farmers, and rural farmers have been trained on these important topics for the development of clean technologies. Interest is increasing among farmers and extension and there is a growing sense of awareness about the potential risks that mishandling steroids pose and the precautions that farmers must take to prevent accidents. All workshops have provided feedback from the attendees that improved the quality of our training material.

### Anticipated Benefits

We have educated students, researchers, extension agents, technicians, and farmers on safe and effective sex inversion techniques in three countries of Central America (Honduras, Guatemala and Costa Rica). The commitment of these personnel is to share the information obtained from us and to train additional producers. A manual in Spanish was distributed to all participants.

### Regional Integration

We exchanged information with Daniel Meyer and Suayapa Meyer, at the Pan-American Agricultural School; Leonel Carrillo at the Centro de Estudios del Mar y Acuicultura from the Universidad de San Carlos de Guatemala; and Juan Ulloa from the Universidad Nacional de Costa Rica at Heredia. We have planned for future collaboration with all scientists from these three education institutions. Five extension agents from the Ministerio de Agricultura y Ganadería of Guatemala (Santiago Yee, Edilberto Ruiz Alvarez, Anaítté Mendez, Glenda Melen-

dez, Goldin Zulema and Melchor Corzo) visited UJAT during April 26–28, 2006 for a hands-on training on sex inversion, production of native cichlids fry, MT elimination from masculinization systems using charcoal filtration and sex identification techniques.

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Kime, D.E., 1998. *Endocrine disruption in fish*. Kluwer Academic Publishers, Boston, Massachusetts, 396 pp.

Schreck, C.B., 1974. Hormonal treatment and sex manipulation in fishes. In: C.B. Schreck (Editor), *Control of Sex in Fishes*. Virginia Polytechnic Institute and State University Extension Division, Blacksburg, Virginia, pp. 84–106.

Table 2. Second Regional Workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture, Cd. de Guatemala, Guatemala. Extension agents, students and small farmers. UJAT-OSU. This workshop took place at Universidad de San Carlos de Guatemala (USAC), Centro de Estudios del Mar y Acuicultura (CEMA) facilities. Decembert 01-02, 2005.

	<b>Name</b>	<b>Institution</b>	<b>Gender</b>	<b>Nationality</b>
1.	Lorena Mérida	Student-CEMA	Female	Guatemalan
2.	Andrea M. Ramirez Aguilar	Student-CEMA	Famale	Guatemalan
3.	Julian Sikahall Prado	Student-CEMA	Male	Guatemalan
4.	Gabriel a. Rivas	Student-CEMA	Male	Guatemalan
5.	Mario Roberto Pinelo	Extension agent	Male	Guatemalan
6.	Cesar Augusto Bolaños	Extension agent	Male	Guatemalan
7.	Antonio Greñas Bonilla	Student-CEMA	Male	Guatemalan
8.	Julio C. Lemus Godoy	Student-CEMA	Male	Guatemalan
9.	Rodrigo Silva	Student-CEMA	Male	Guatemalan
10.	Hugo Siliezar Mendizabal	Student-CEMA	Male	Guatemalan
11.	Luis E. Quiñones	Student-CEMA	Male	Guatemalan
12.	Javier Gabriel Quiroa	Student-CEMA	Male	Guatemalan
13.	Estrella Marroquín Herrera	Researcher-CEMA	Female	Guatemalan
14.	Daniel Alejandro Castro	Student-CEMA	Male	Guatemalan
15.	Alan Humberto Herrera	Student-CEMA	Male	Guatemalan
16.	Jorge Mario Ruano	Student-CEMA	Male	Guatemalan
17.	Katia Iturbide Dormon	Student-CEMA	Female	Guatemalan

## APPLIED TECHNOLOGY AND EXTENSION METHODOLOGIES

Table 3. Third Regional Workshop, Safe Handling of Steroids and Clean Technologies in Aquaculture. Heredia, Costa Rica. Extension agents, students and small farmers. UJAT-OSU. This workshop took place at Universidad Nacional (UNA). Escuela de Ciencias Biológicas, Campus Omar Dengo facilities. June 22-23, 2006

	Name	Institution	Gender	Nationality
1.	Carlos Alvarado R.	Farmer Private-ACUACORPORACION	Male	Costa Rican
2.	Douglas Elizondo S.	Farmer Private-ACUACORPORACION	Male	Costa Rican
3.	Muricio Castro V.	Farmer Private-El Pelon de la Bajura	Male	Costa Rican
4.	Guillermo Gómez C.	Farmer Private-COOPEBATAAN	Male	Costa Rican
5.	Gerardo Zamora O.	Extension agent-INOPECA-Guapiles-Government	Male	Costa Rican
6.	Edagar Chacon P.	Extension agent-INOPECA-San Carlos-Government	Male	Costa Rican
7.	Efrain Duran S.	Extension agent-INOPECA-Cañas-Government	Male	Costa Rican
8.	José L. Piña A.	Farmer Private-La Pacifica	Male	Costa Rican
9.	Adrian Sevilla	Researcher-Univeridad Nacional	Male	Costa Rican
10.	Jorge Boza A.	Researcher-Univeridad Nacional	Male	Costa Rican
11.	Silvia Valverde Ch.	Researcher-Univeridad Nacional	Female	Costa Rican
12.	Nazira Gálvez H.	Researcher-Univeridad Nacional	Female	Costa Rican
13.	Jonathan Chacon	Parque Marino Government-Universidad	Male	Costa Rican
14.	Martín Méndez H.	Parque Marino Government-Universidad	Male	Costa Rican
15.	Norman Salas R.	Farmer Private-Valle Cocodrilo S.A.	Male	Costa Rican
16.	Roy Salas E.	Farmer Private-Valle Cocodrilo S.A.	Male	Costa Rican
17.	Joaquín Calderón V	Escuela Ag. de la Región Trópico Húmedo (EARTH)	Male	Costa Rican
18.	Alex Molina Arias	Instituto Costarricense Electricidad	Male	Costa Rican
19.	Onelio López R.	Aguilar y Solís-Alimentos	Male	Costa Rican
20.	Ronny Cordero S.	Escuela Centroamericana de Ganaderia	Male	Costa Rican
21.	Cristian Alvarado A.	Mostes de Oro-Alimentos	Male	Costa Rican
22.	Karol Ulate N.	Student-UNA	Female	Costa Rican
23.	Daniel Velarde A.	Student-UNA	Male	Costa Rican
24.	Karen Berrocal	Student-UNA	Female	Costa Rican
25.	Miguel Brais M.	Student-UNA	Male	Costa Rican
26.	Guillermo Zúñiga R.	Student-UNA	Male	Costa Rican
27.	Luis Hernández	Student-UNA	Male	Costa Rican
28.	Félix Carranza C.	Government-Ministerio de Agricultura y Ganaderia	Male	Costa Rican
29.	Gilbert Contreras	Government-Ministerio de Agricultura y Ganaderia	Male	Costa Rican
30.	Juan Ulloa	Researcher-Universidad Nacional	Male	Costa Rican



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## ESTABLISHMENT OF THE CENTER FOR AQUACULTURE TECHNOLOGY TRANSFER

*Twelfth Work Plan, Applied Technology and Extension Methodologies 5 (12ATE5)  
Abstract*

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### ABSTRACT

The Centro de Transferencia Tecnológica Para La Acuicultura (CETRA) has been established at Universidad Juárez Autónoma de Tabasco in Mexico (UJAT). The Center includes a consortium of Mexican universities and public/private foundations that have an associated interest in aquaculture. Its goal is to support and guide aquaculture commercial enterprise development in an environmentally sustainable fashion. CETRA has established the initial network of academic and economic resources in Mexico and the United States that provide extension services for meeting Mexico's sustainable aquaculture development goals. A website has been created towards this end: [www.cetra.org.mx](http://www.cetra.org.mx)

CETRA has elected its first director (Dr. Eunice Perez Sanchez of UJAT), its first co-director (Dr. Margarita Cervantes Trujano of ITBOCA), and developed its mission statement.

#### CETRA Mission Statement:

Administer sustainable aquaculture technology transfer to the Mexican private sector through an inter-institutional collaborative network that encompasses all phases of the process.

The center has built upon the research, extension, and outreach efforts recently made by the CRSP/USAID programs on the east and west coasts of Mexico and the summer 2004 extension meeting of US and Mexican universities of the Gulf of Mexico. It supports the ACRSP program area of Production Technology in a way that patterns the US Sea Grant program. CETRA networks over 15 universities and institutions throughout Mexico through its centrally based website and director. It is focusing initially on the single theme of sustainable aquaculture production.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## SPECIAL SESSIONS, TRAVEL, AND POSTER AWARDS AT 2005 AND 2006 WORLD AQUACULTURE CONFERENCES AND AQUACULTURE AMERICA 2006

*Twelfth Work Plan, Applied Technology and Extension Methodologies (12ATE9)  
Final Report*

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Remedios Bolivar  
Central Luzon State University  
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### **ABSTRACT**

Aquaculture CRSP (ACRSP) sessions were organized and conducted at the US Aquaculture meetings in Las Vegas NV in February 2006, and at the World Aquaculture Meetings in Bali, Indonesia and Florence, Italy in May 2005 and May 2006, respectively. In addition, funds were used to support participation of four host country scientists to attend the Southern Africa Aquaculture Conference in September of 2005, which also had an ACRSP session. Each of these sessions was well attended and included a full compliment of presentations of A CRSP sponsored research. In conjunction with these sessions, travel awards were provided to at least three scientists involved in the research who were not able to otherwise attend the conference on project funds. The travel awards were determined on merit; depending on contribution to the research, quality of the abstract, participation in earlier A CRSP sponsored research and quality of the Power Point presentation developed for the conference.

A second aspect of this project was a series of awards for student posters presented at the same above mentioned conferences, as well as the US Aquaculture Meetings in New Orleans in 2005. Three awards, plus cash certificates, were presented to the top three student poster presenters. The posters were judged on scientific quality, contribution to the core ACRSP principle of sustainable aquaculture practices, and appearance and use of graphics. Publicity of the awards was wide spread with photos appearing in newsletters, aquaculture industry magazines, and various websites.

The project has been successful by improving recognition of the quantity and quality of research supported by the ACRSP. Much of the industry recognized and appreciated work done by many leading aquaculture scientists but had been unaware that the ACRSP was a primary sponsor. By organizing these specific sessions and awards, the contributions of the ACRSP and US-AID sponsorship have been much more widely recognized.

## INTRODUCTION

The Aquaculture CRSP has a long history of supporting travel to WAS conferences where research results were presented. However, recognition of the ACRSP as a funding and support entity were lacking due to the diffuse presentations across many disciplines within aquaculture. By organizing specific sessions for presentation of ACRSP results the recognition of the ACRSP was increased by an order of magnitude. Further, also providing travel support and recognizing student posters with awards further magnified the scope of ACRSP contributions.

## RESULTS

### Pre-Conference Professional Awards

World Aquaculture Society, Florence, Italy

The Aquaculture CRSP provided numerous pre-conference professional awards associated with the World Aquaculture Society conferences in Bali Indonesia, (May 2005), and Florence, Italy (May 2006), and the Southern Africa Aquaculture Meetings in Grahamstown, S.A. (September 2005). These awards are typically provided to young professionals and awarded to individuals based upon the following criteria:

1. Providing a presentation during the conference related to past or present Aquaculture CRSP research.
2. Scientific merit based upon submitted abstract.
3. Demonstrated financial need and matching leverage funds.
4. Regional distribution of research results.

Awardees for the 2005 Bali Pre-Conference Professional Awards included:

Lourens de Wit, Stellenbosch University, South Africa  
Lai Qiuming, Hainan University, Hainan, China  
Khalid Salie, Stellenbosch University, South Africa  
Agus Somamihardja, Asian Institute of Technology, Thailand

Awardees for the 2005 South Africa Pre-Conference Professional Awards included:

Kajitanus Osewe, Department of Fisheries, Tanzania  
Charles Ngugi, Moi University, Kenya  
Mucai Muchiri, Moi University, Kenya

Awardees for the 2006 Florence Pre-Conference Professional Awards included:

Guillermo Rodriguez, Universidad Autonoma de Sinaloa, Mexico  
Murat Arslan, Ohio State University, USA  
Khalid Salie, Stellenbosch University, South Africa  
Md. Asaduzzaman, Bangladesh Agricultural University, Bangladesh

(Note: Unfortunately Md. Asaduzzaman, never received an Italian visa and was unable to attend the conference.)

### Student Poster Awards:

World Aquaculture Society, Bali, 2005

The Aquaculture CRSP sponsored three student poster awards at the WAS Annual Conference in Bali Indonesia in May 2005. One first place and two runner-up awards were given to those posters judged as the best representatives of the broad research and development theme "to advance sustainable aquaculture." All posters submitted by students were considered and judging

was conducted using accepted Aquaculture CRSP guidelines. Specific judging criteria focused on value of the contribution to sustainable aquaculture development, technical quality of the study and level of involvement required, presentation and use of graphics, and overall applicability and benefits of the results.

Aquaculture CRSP awards were presented to the winning students during the Student Reception of the WAS Bali conference. The highest judged student posters at the World Aquaculture Society 2005 meeting were as follows:

#### First Place:

Charlie Price, University of Stirling, Scotland. Effects of pesticide residues on vegetable grown in ditch-dyke systems and implementation of pesticide minimization experiments in Central Thailand.

#### Runners-up:

Shanti Dwita Lestari, Bogor Agricultural Institute, Indonesia. Water quality of aquaculture sites using *Lactobacillus sp.* as a probiotic microbial species.

Cristian Gallardo-Escarate, Centro de Investigación Científica y de Educación Superior de Ensenada, México. Karyotype análisis and chromosomal localization by fish rDNA, telomeric (TAAGGG)<sub>n</sub>, and (GATA)<sub>n</sub> repeats in *Haliotis fulgens*.

*Aquaculture America, New Orleans, 2005*

The Aquaculture CRSP sponsored three student poster awards at the Aquaculture America 2005 meeting in New Orleans. One first place and two runner-up awards were given to those posters judged as the best representatives of the broad research and development theme "to advance sustainable aquaculture." All posters submitted by students were considered and judging was conducted using accepted Aquaculture CRSP guidelines. Specific judging criteria focused on value of the contribution to sustainable aquaculture development, technical quality of the study and level of involvement required, presentation and use of graphics, and overall applicability and benefits of the results.

Aquaculture CRSP awards were presented to the winning students during the USAS Student Reception. In addition, Dr. James Avault kindly donated a copy of his book "Fundamentals of Aquaculture: A Step-by-Step Guide to Commercial Aquaculture" to each of the winning students. The highest judged student posters at Aquaculture America 2005 were as follows:

#### First Place:

Peng Li, Department of Wildlife and Fisheries Sciences, Texas A&M University. Evaluation of the prebiotic Grobiotic-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass *Morone chrysops* X *M. saxatilis* challenged in situ with *Mycobacterium marinum*.

#### Runners-up:

Joseph Sawdy, Department of Animal Sciences, The Ohio State University. Whole muscle 1-D protein fingerprints of yellow perch *Perca flavescens* correlate with total body weight and length.

Roberto Quintana, Aquaculture Research Station, Louisiana State University. Rapid estimation of gonad-to-body ratio in oysters.

*US Aquaculture, Las Vegas, NV, 2005*

**First Place:**

Jamie Green, Kentucky State University. Impact of substrate color, material, surface area and mesh size on survival and growth of freshwater prawn *Macrobrachium rosenbergii* reared in pond microcosm tanks.

**Runners-up:**

Warren Jones, University of Alabama at Birmingham. Dietary requirements for the sea urchin *Lytechinus variegates*

Kyle Schneider, Kentucky State University. How does length of the nursery period effect subsequent pond production of freshwater prawns.

*World Aquaculture Society, Florence, Italy, 2006*

The Aquaculture CRSP sponsored three student poster awards at the WAS Annual Conference in Florence Italy in May 2006. One first place and two runner-up awards were given to those posters judged as the best representatives of the broad research and development theme "to advance sustainable aquaculture." All posters submitted by students were considered and judging was conducted using accepted Aquaculture CRSP guidelines. Specific judging criteria focused on value of the contribution to sustainable aquaculture development, technical quality of the study and level of involvement required, presentation and use of graphics, and overall applicability and benefits of the results.

Aquaculture CRSP awards were presented to the winning students during the Closing Ceremony of the WAS Florence conference. The highest judged student posters at the World Aquaculture Society 2006 meeting were as follows:

**First Place:**

M. Ekram Zsim, University of Stirling, Scotland. Production of microbial protein using active suspension technique (AST) in artificial ponds.

**Second Place:**

Mahanama De Zoysa, Cheju National University, Republic of Korea. Isolation and purification of bioactive polysaccharide as an antiocagulant from edible fermented brown seaweed *Sargassum fulvellum*

P.R. Battle, Virginia Tech University. A recirculating life support system for the propagation of hard corals to support the aquarium, biomedical and reef rehabilitation industries.

### DISCUSSION AND CONCLUSIONS

The project has successfully brought eleven scientists from host countries to participate in the World Aquaculture or the South Africa meetings. This has provided a direct boost to their professional careers and helped to disseminate information from the professional presentations at the WAS back to the host countries.

The students' awards provided very good publicity to the hard work of many deserving students and their education programs. Further, they provided a centerpiece to the student functions at the meetings and served to raise the profile of the student posters.

The A CRSP sessions themselves served to bring together ACRSP sponsored research that in the past had been delivered across many sessions. The higher visibility of the A CRSP has been important recognition of the scope of A CRSP work and the support provided by the US AID.

### ANTICIPATED BENEFITS

Some of the immediate benefits from the pre-conference awards were the rapid dissemination of new information presented by the participants both in the oral and social portions of the WAS. On a longer term basis, the knowledge gathered at the WAS meetings that returned with the participant to the host country is an important aspect of improving capacity.

There are several benefits for the students who receive the poster awards. The cash awards are very helpful to the students, who are usually operating with limited means. However, the professional recognition is the real benefit. This achievement is an important line on a CV or resume that will help them to move to new positions and jobs in their careers.

Finally, a true benefit for the A CRSP is the publicity and recognition that the A CRSP garners as a key contributor to research in the aquaculture field. The awards and special sessions served to raise the profile of the A CRSP by focusing the array of A CRSP work.

### ACKNOWLEDGMENTS

A large number of senior ACRSP scientists and administrators served as judges for the student poster awards. This entailed many hours of carefully reading a large number of posters during a very busy time when there were competing demand on their time. Judging is under-recognized as an important professional contribution that carries little prestige but is critical to the success of scientific programs.

Several senior ACRSP scientists and administrators also served to judge the pre-conference awards. Again their contributions are critical the program and under-recognized.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## AQUACULTURE CRSP SPONSORSHIP OF THE SEVENTH INTERNATIONAL SYMPOSIUM ON TILAPIA IN AQUACULTURE

*Twelfth Work Plan, Applied Technology and Extension Methodologies 10 (12ATE10)  
Final report*

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### ABSTRACT

The Seventh International Symposium on Tilapia in Aquaculture (ISTA7) was held from Sept 6–10, 2006 in Veracruz, Mexico. Aquaculture CRSP was one of the co-sponsors for the symposium. The ACRSP has also been a co-sponsor of two earlier ISTA's in Brazil and the Philippines. Two books were published in conjunction with the Seventh ISTA; the proceeding of ISTA7, and an Atlas of Tilapia Anatomy and Normal Histology. ACRSP was the co-publisher of both these volumes. Sixty-seven papers were accepted for presentation and inclusion in the proceedings. ACRSP contributed to the publication of 1000 copies of each of the books. More than half the proceedings were distributed to the participants and 100 copies were given to Universidad Juarez Autonoma de Tabasco for use in their programs and 100 copies were provided to Panorama Acuicola for distribution to subscribers of their magazine. The final day of the symposium was devoted to farm tours highlighting small-scale tilapia farms in the state of Veracruz, Mexico.

In addition to the main conference, a Recirculating Aquaculture Workshop was held for three days immediately prior to the ISTA7. This workshop had 117 registered participants. The ACRSP ISAT7 project also supported travel and participation in the ISTA 7 from two project Principal Investigators, (Contreras from Mexico and Bolivar from the Philippines). The project also supported travel and participation of five Mexican graduate students to participate in ISTA7.

### INTRODUCTION

The ACRSP has co-sponsored several of the ISTA conferences. The Seventh ISTA was based on the highly successful sixth symposium. The Sixth International Symposium on Tilapia in Aquaculture (ISTA6) was held 12–16 September 2004 at the Philippines International Convention Center in Manila, Philippines. It brought together experts in tilapia growing to review the latest discoveries in tilapia biology, ecology, improvements in production systems, and other fields related to tilapia in aquaculture. The forum was an opportunity to display the Aquaculture CRSP's long involvement in tilapia research, which was evident from the number of presentations made by the group in ISTA6 covering different aspects of tilapia research. The group for the joint research conducted by the Aquaculture/CRSP and the Asian Institute of Technology (AIT) made a total of 15 scientific presentations.

Yang Yi delivered a talk on tilapia culture in China jointly with Lai Quiming of Hainan University, China, during the plenary. They presented an extensive review on the history and current status of tilapia culture in Mainland China and a critical analysis of different components of tilapia aquaculture in China, particularly in light of the outstanding Chinese success in registering 25% annual growth in tilapia production between 1987 (18,100 metric tons) and 2002 (706,585 metric tons). They

observed that though China is the largest producer of tilapia in the world, the production is quite localized, as the top-five tilapia producing provinces accounted for 87.5% of the total tilapia production in China in 2002. Amrit Bart, Coordinator of AARM at AIT, presented on AARM contributions to tilapia research. In his presentation he acknowledged the strong cooperation between the Aquaculture CRSP and AIT in conducting joint research on tilapia, which resulted in several valued publications in peer reviewed journals. He noted that joint research conducted by Aquaculture CRSP and AIT to optimize tilapia production by improving primary production and husbandry practice during 1980s became a theme that continues today at AARM, AIT. C. Kwei Lin presented two papers related to red tilapia culture in brackishwater. He observed that red tilapia grew better in brackishwater than in the freshwater, and the best growth performance was achieved at 10 ppt salinity. He also found that a fertilization system 50% satiation feeding was the most efficient feeding rate to grow red tilapia. M. K. Shrestha presented two papers, one on control of Nile tilapia recruitment by predatory snakehead and second on grass carp Nile tilapia polyculture. His research findings showed that snakehead were able to control Nile tilapia recruitment completely and thus, may provide an alternative technique for Nile tilapia culture. D. P. Thakur presented research on fertilization



and feeding strategy for Nile tilapia grow-out ponds, and concluded that combination of fertilization and feeding should be a preferred strategy over fertilization followed by feeding for culturing Nile tilapia.

Yang Yi was the leading presenter in the group with four oral and four poster presentation in ISTA6. Details for all the presentations made by the group can be found in the ISTA6 proceeding as manuscript for all the presentations was published in the Symposium proceeding; ISTA6 published two volumes of proceedings edited by Remedios Bolivar of Central Luzon State University, Kevin Fitzsimmons of the University of Arizona, and Graham Mair of Finders University. The list of all the presentations made by the group in ISTA6 is shown below.

#### Presentations:

- Amrit Bart, contribution of Aquaculture and Aquatic Resources Management (AARM) Program of the Asian Institute of Technology (AIT) to tilapia research.
- Yang Yi, Stocking densities of Nile tilapia in shrimp ponds under different feeding strategies.
- C. Kwei Lin, effects of fertilization rates on growth performance of red tilapia at different salinities.
- C. Kwei Lin, supplemental feeding for red tilapia culture in brackishwater.
- Yang Yi, stocking densities and fertilization regimes for Nile tilapia (*Oreochromis niloticus*) production in ponds with supplemental feeding.
- Yang Yi, stocking ratios of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) and Nile tilapia (*Oreochromis niloticus*) in an intensive polyculture.
- D. P. Thakur, effects of fertilization and feeding strategy on water quality, growth performance, nutrient utilization and economic return in Nile tilapia (*Oreochromis niloticus*) ponds.
- M. K. Shrestha, culture of mixed-sex Nile tilapia with predatory snakehead.
- M. K. Shrestha, polyculture of grass carp and Nile tilapia with napier grass as the sole nutrient input in the subtropical climate of Nepal.
- Yang Yi, recycling wastewater of intensive hybrid *Clarias* catfish culture for semi-intensive Nile tilapia culture.
- Yang Yi, management of organic matter and nutrient regeneration in pond bottoms through polyculture.
- Yang Yi, tilapia-shrimp polyculture in Thailand.
- Yang Yi, effects of adding shrimp (*Penaeus monodon*) into intensive culture ponds of Nile tilapia (*Oreochromis niloticus*) at different densities.
- Yang Yi, tilapia culture in China.
- N.T. Phuong, integrated cage-cum-pond culture: stocking densities of caged climbing perch in Nile tilapia ponds.

#### MATERIALS AND METHODS

ACRSP scientists provided the bulk of the organization and planning for the ISTA7 conference. UJAT and UA developed websites for the symposium. The UJAT site included a database for receiving abstracts and full papers that was eventually used to develop the conference proceedings book. UA worked with Panorama Acuicola magazine to procure \$100,000 in matching funds from the State of Veracruz to support the conference. UJAT helped to coordinate the pre-conference work-

shop and provided students to assist the conference logistics. UA provided a vehicle for logistical support as well as bringing conference materials, laptop computers, and projectors.

The Veracruz World Trade Center was selected as the venue with a number of large and small conference rooms and trade show hall capable of holding 20 meter round production tanks along with the rest of the vendors' equipment and tanks. Several tanks and aquaria were stocked with tilapia. One large tilapia producer also displayed several live crocodiles that are reared in conjunction with the tilapia farm.

#### RESULTS

The Seventh International Symposium on Tilapia in Aquaculture was recently completed in Veracruz, Mexico. The workshops, trade show, technical presentations, farm tours and tilapia dinner were attended by a total of 971 individuals. This was by far the most successful of any of the ISTA conferences and demonstrated the rapid growth of tilapia aquaculture as a global industry. ISTA7 was organized by US and Mexican scientists from the Aquaculture CRSP and Aquaculture TIES projects, both supported by US-Agency for International Development, and their partners from Panorama Acuicola Magazine based in Mexico. The symposium was hosted by the Government of Veracruz and the Governor, Fidel Herrera Beltran, provided the opening address. The governor's address included a description of the rapid growth of aquaculture, especially tilapia, in the state of Veracruz and announced a new package of investments in further support of aquaculture education, research, and production for the state.

The main conference was actually preceded by a three-day workshop on recirculating aquaculture systems attended by 117 participants. This workshop was also sponsored by a separate Aquaculture CRSP grant and was conducted by a team including Drs. Mike Timmons, Raul Piedrahita and James Ebeling. They were assisted by Drs. Margarita Cervantes Trujano and Eunice Perez-Sanchez, from the IT-Boca and UJATabasco, respectively. Dr. Dallas Alston, from the University of Puerto Rico provided an excellent keynote address, "global outlook of tilapia aquaculture with emphasis on Latin America". Concurrent sessions over the next two and half days covered the entire field of tilapia aquaculture with presentations on reproduction and genetics, pathology, nutrition, production systems, and processing and markets. A trade show held in conjunction was well attended and included most of the major suppliers of aquaculture equipment, feeds, and fingerlings.

During the conference three new reference books debuted with strong sales at an author's signing party (Figure 1). "Atlas of Tilapia Histology", co-published by the Aquaculture CRSP and World Aquaculture Society, was presented by one of the co-authors, Kevin Fitzsimmons. "Tilapia: Biology, Culture, and Nutrition", published by the Haworth Press was presented by one of the co-editors, Chhorn Lim and "Tilapia Culture" published by CABI was presented by the author, Dr. Abdel-Fattah El-Sayed, from Alexandria University in Egypt.

The Tilapia International Foundation also presented the Jan Heijne Memorial Award to ISTA7 Principal Investigator, Kevin Fitzsimmons in recognition of his lifetime contributions to tilapia aquaculture. The award was presented by Jan-Jaap vande Velde, the Dutch Ambassador to Mexico (Figure 2).



Figure 1. Dr. Abdel-Fattah El-Sayed (left) and Dr. Kevin Fitzsimmons (right) present their books at the author's signing party.

A gala tilapia dinner was provided on the third night of the ISTA, with ten restaurants each providing two tilapia dishes, which the delegates were invited to try. The delegates were invited to promenade past the restaurant booths prior to selecting their dinner, according to Veracruzana tradition accompanied by mariachis and local folk dancers.

On the final day of the symposium a farm tour visited four farms in close vicinity or even with the city of Veracruz. These ranged from a large industrial farm focused on international sales, to intermediate farms for local sales, to a small farm integrated to a restaurant chain that sells all their products, including providing a set lunch to the touring ISTA delegates.

### DISCUSSION AND CONCLUSIONS

The conference was widely reported to be a great success. Television, radio, newspaper, and internet coverage were extensive. The proceedings (Tilapia, Sustainable Aquaculture from the New Millennium (W. Contreras-Sanchez and K. Fitzsimmons, Editors). Proceedings of the Seventh International Symposium on Tilapia in Aquaculture, American Tilapia Association, Aquaculture CRSP 389 pp.) were widely distributed and are now for sale at the WAS website. There were 971 registered participants and the Governor of Veracruz has begun lobbying to host the 2009 World Aquaculture meetings because of the local government's satisfaction with the positive outcomes from the ISTA. Egypt and Taiwan have both requested consideration for hosting a future ISTA.

### ANTICIPATED BENEFITS

Some of the immediate benefits from ISTA7 were the rapid dissemination of new information presented by the participants both in the oral and social portions of the symposium and the written proceedings. Further, three new books debuted at the meeting with strong sales. An equally important benefit was the additional funding to support aquaculture from the State of Veracruz. A longer-term benefit is increased demand for tilapia generated by increased sales through the Veracruz restaurants and grocery stores. The publicity from the participants' consumption of several thousand pounds of tilapia increased local demand of tilapia, and this publicity should continue indefinitely. Many of the producers in attendance reported that they had acquired considerable knowledge that they would apply at their individual farms.

### ACKNOWLEDGMENTS

Sources of funding other than the ACRSP included the State of Veracruz, Aquaculture TIES project, American Tilapia Association, and the Tilapia International Foundation.

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Morrison, C., K. Fitzsimmons, and J.R. Wright, 2006. Atlas of Tilapia Histology. World Aquaculture Society, Baton Rouge, LA., 96 pp.



Figure 2. Dr. Kevin Fitzsimmons (right) receives the Jan Heijne Memorial Award.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## **KENYA TRAINING-OF-TRAINERS AND REGIONALIZATION OF AQUACULTURE TRAINING ACTIVITIES**

*Twelfth Work Plan, Applied Technology and Extension Methodologies 11 (12ATE11)*  
*Abstract*

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### **ABSTRACT**

This activity addresses new (2005–2006) objectives for the OSU/Kenya project, including conducting additional training courses (one training-of-trainers course for Fisheries Officers followed by two pond construction and management courses for fisheries assistants, taught by the new trainers) in Kenya and providing support for the participation of Kenyan PI Charles Ngugi in training courses held in other countries in the region.

The intent of training trainers in Kenya is to increase the number of individuals who can effectively teach basic pond construction and management techniques to extension agents and farmers. It is expected that 10 Fisheries Officers will be selected to be trained as trainers and that they will then conduct two further short courses for up to 15 Fisheries Assistants each.

Supporting the participation of Charles Ngugi in training courses in Tanzania, Ghana, or other countries is intended to make the experiences and materials developed by the Kenya Project available for training efforts in the region as a whole, as well as to encourage regional collaboration in aquaculture training efforts. Ngugi is skilled in the practical aspects of pond construction and pond and hatchery management and he has a teaching style that makes the subject matter highly accessible to trainees, whether they are farmers or extension workers. He has previously traveled throughout the region and his fluency in both Swahili and English will enable him to easily fit in and make valuable contributions to training courses in other countries. These activities are scheduled to take place between September 2006 and April 2007.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## POST-TSUNAMI TRAINING IN NEW AQUACULTURE TECHNOLOGIES IN THAILAND AND INDONESIA

*Twelfth Work Plan, Applied Technology and Extension Methodologies 14 912 ATE14)  
Final Report*

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### ABSTRACT

Coastal aquaculture was one of the primary occupations in Aceh province, Indonesia prior to the tsunami of December 26, 2004. Over 30,000 families listed aquaculture as their primary means of income and/or subsistence. When the tsunami struck many of these people lost lives and had their ponds destroyed. While the relief efforts did a tremendous job of feeding and housing survivors, the restoration activities of rebuilding the economy and especially of restoring aquaculture facilities have been slow.

Principal investigators from the Asian Institute of Technology and University of Arizona collaborated with Aquaculture without Frontiers, other NGOs, and the Indonesian Department of Fisheries to conduct a series of workshops, supply materials to the fisheries/aquaculture school, contribute materials and expertise to pond restorations, and provide guidance by e-mail to further these efforts. Specifically, three workshops were conducted in various locations in Banda Aceh, two collections of text and reference books were purchased and delivered to the Ladong Fisheries College and the Ujong Batee Aquaculture Research and Extension Center, technical advice was provided for three restoration efforts to convert shrimp hatcheries to multi-species facilities working with seaweeds, tilapia, other invertebrates as well as shrimp.

All parties have agreed in principle that a system of sustainable coastal aquaculture is desirable to follow for restoration of the considerable aquaculture sector in the province. The Aquaculture Collaborative Research Support Program was and is uniquely qualified to provide needed expertise and guidance towards developing the sustainable coastal aquaculture that will be needed to achieve a restoration effort that will effectively raise the standard of living in Aceh.

### INTRODUCTION

On December 26, 2004 at 6:58 a.m. (local time), a 9.0 magnitude earthquake occurred in deep water off the western coast of Northern Sumatra, Indonesia. The earthquake triggered a massive tsunami. Large waves struck without warning and severely impacted the coasts of South and Southeast Asia, including Indonesia, Thailand, Sri Lanka, India, stretches of the Malaysian coast and many small islands in the Andaman Sea.

The 26 December tsunami devastated Thailand's entire Andaman coast strip. A total of 392 villages and some 54,500 people were affected by the tsunami, with more than 5,000 deaths recorded and many others missing. Over 4,500 fishing boats were destroyed or seriously damaged. Across the Andaman coast, tens of thousands are without jobs, income, have dwindling means of survival, and few if any alternatives. Residents want jobs and to get back with their lives.

With this sudden impact on fisheries and other resource dependent livelihoods, the need to restart and diversify livelihoods is

acute. In the long run, livelihood development creates disaster resilient communities because communities with diverse economic bases are not dependent on just one activity.

New and diversified livelihoods mean introducing new production technologies to coastal villages. Aquaculture offers an alternative livelihood with potential for expansion in the project site. This study explores the openness of aquaculturists and others to adopt new catfish hatchery technology that would permit an expansion in catfish culture in the selected communities. We will also provide training in other aquaculture crops believed to provide diversity and more sustainable coastal aquaculture compared to the monoculture of shrimp in ponds. Following the tsunami disaster, thousands of fishers are without jobs. This study will also examine the openness of fishers to be part time fish farmers, thereby reducing overexploitation of the fisheries and livelihood dependence on the wild capture fishery.

The proposed study builds on and would be supported in part by the USAID Post-Tsunami Sustainable Coastal Livelihoods Program, a model program to demonstrate sustainable coastal communities that are resilient to economic and environmental shocks. The goal of the program is to rebuild and diversify sustainable coastal livelihoods of severely affected fishing communities on the Andaman Coast of Thailand and to demonstrate effective practices of community-based disaster preparedness. The Program is implemented by the Coastal Resources Center of the University of Rhode Island in partnership with the Asia Institute for Technology (AIT), University of Hawaii, and other local partners.

The World Aquaculture Society Tsunami Relief Fund, the Ujong Batee Aquaculture Center, and Aquaculture without Frontiers would jointly support the Indonesian aspects. Direct cash match of \$10,000 would come for the WAS fund. Additional in-kind contributions would come from Professionals International and Aquaculture without Frontiers, delivering course materials, providing transportation and assisting with translations.

This award to URI was made through the Sustainable Coastal Communities and Ecosystems (SUCCESS) Cooperative Agreement with USAID. This study, therefore, also builds working relationships between SUCCESS and ACRSP.

### MATERIALS AND METHODS

On evening of March 8, 2006, Kevin Fitzsimmons departed Tucson, bound for Banda Aceh, with arrival scheduled for March 11. The overall goal of the project was to contribute to the restoration of aquaculture in Aceh Province by capacity building and directing the restoration efforts toward a sustainable coastal aquaculture methodology rather than a return to monoculture of shrimp in mangrove estuaries. The specific objectives of the trip were to:

- 1) Conduct two workshops in the tsunami impacted zone of Aceh province directly with artisanal shrimp farmers.
- 2) Conduct a third workshop for representatives from NGOs, FAO, Indonesian Department of Fisheries staff, and faculty and students from Ladong Fisheries College.
- 3) Visit three hatchery restoration projects partially supported by Aquaculture without Frontiers, the aquaculture charity/NGO, to observe and consult on progress and future efforts.
- 4) To deliver donated textbooks, reference materials, laboratory materials and field equipment to the Ladong Fisheries College and the Ujong Batee Aquaculture Research and Extension Center.
- 5) Visit the Tibang village restoration site supported by Mercy Corps and discuss future options for aquaculture pond restoration with Mercy Corps representatives.

### RESULTS

Three workshops were presented in Banda Aceh.

Title: Sustainable coastal aquaculture for restoration (Figure 1).

Location: Fisheries College at Ladong

Date: March 12, 2006

Number of individuals trained: 60

Individuals involvement: We used PowerPoint to present topics, photographs, and initiate discussions. Farmers participated in discussions, were introduced to NGO and governmental experts, and each received a seaweed growing handbook.



Figure 1. Participants of the sustainable coastal aquaculture for restoration workshop at the Fisheries College at Ladong.

Title: Sustainable coastal aquaculture for restoration

Location: Village mosque in Samalanga

Date: March 13, 2006

Number of individuals trained: 52

Individuals involvement: We used PowerPoint to present topics, photographs, and initiate discussions. Farmers participated in discussions, received a box lunch and met governmental experts from Ujong Batee. Each farmer received a seaweed growing handbook and were invited to accompany is to visit a group fingerling farm.



Figure 2. Participants of the sustainable coastal aquaculture for restoration workshop at the village mosque in Samalanga.

Title: Sustainable coastal aquaculture for restoration (Figure 3).

Location: Aquaculture research and extension center at Ujung Batee

Date: March 13, 2006

Number of individuals trained: 50

Individuals involvement: We used PowerPoint to present topics and photographs and initiate discussions. Farmers and NGO reps participated in discussions, were introduced governmental experts, and each received a seaweed growing handbook.

DISCUSSION AND CONCLUSIONS



Figure 3. Participants of the sustainable coastal aquaculture for restoration workshop at the aquaculture research and extension center at Ujung Batee.

Each workshop included a presentation reviewing the pre-tsunami status of shrimp farming including disease problems, effluent discharge issues, removal of mangroves, and declining yields and prices for shrimp. We then provided information on more sustainable methods of coastal aquaculture including diversification of products to include seaweeds, tilapia, milkfish, grouper, sea cucumbers, and bivalves. We discussed the ecology of coastal production systems and the need to improve pond dynamics. We discussed the current market conditions for shrimp and the other products we were promoting for consideration. Discussion ensued regarding the supplies to existing markets and how to reach or develop new markets for proposed crops. We provided an illustrated seaweed culture book by Glenn et al. to each participant to assist the training efforts. Another important discussion was the role of government, NGOs, and personal initiative in restoration efforts. Much of our presentation dealt with the capacity of small farmers to incorporate seaweed, fish, and bivalve culture with minimal resource inputs. Greater investment is required for shrimp culture, and many of the farmers told us that they would start with the polyculture concept, as they could not continue to wait for government or NGO support to restart shrimp farming. Finally we discussed the need for mangrove restoration and shared the contact points for obtaining juvenile mangroves from nurseries operated by several NGOs in the region.

In the afternoons we visited the hatchery restoration projects supported by AwF with donations from Yellow Springs Instruments (YSI and its employees). The hatcheries had been supplied with new roofs, water and air pumps and restoration of electrical supply. One of the hatcheries had post-larvae almost ready for sale to local farms. Another had water flowing, new broodstock on site and Artemia in hatch tanks preparing spawning events. The third hatchery had roof and equipment installed, but did not have any animals on site and was still some days from operational capacity.

On the last afternoon we visited the pond restoration project in Tibang supported by Mercy Corps. We viewed the pond work and made several suggestions to the on-site manager to alter some of the earthwork to provide for better water flow and incorporate part of the mangrove plantings as biofilters for pond effluent.

Farmers and NGOs reported that they were anxious to implement various aspects that were presented and appreciated the supporting documentation provided directly to them as well as the volumes of technical documentation left with the research center and the school. Most of those attending shared with us that they felt this was the path they would take in hopes of developing sustainable aquaculture operations in Aceh and avoid the downward spiral that had impacted them prior to the tsunami.

The project received support from the Aquaculture CRSP, Aquaculture without Frontiers, and logistical support (housing, transportation) from the Ujung Batee Aquaculture Research Center, and organizational support for workshops from Professionals International.

The list of representatives from NGOs attending included Mercy Corps, Professionals International, World Wildlife Fund, and the French and American Red Cross. Also in attendance were representatives from the Fisheries Department of Indonesia and UN-FAO

As part of the follow-up, we have been invited by the Rockefeller Foundation to host a meeting at the Bellagio Conference Center in Italy to continue to move the process forward. Of special interest is development of a sustainable aquaculture plan that could be presented to aid organizations that have withheld restoration funds until environmental groups are satisfied that aquaculture will not revert back to unsustainable methods such as monoculture of marine shrimp.

Anticipated Benefits

Many of the farmers we met reported that they were going to incorporate our suggestions in their restoration plans. The Ujung Batee staff and Ladong School faculty also reported that they would incorporate these concepts in their extension and teaching, respectively. Since the workshops, the government has also provided seaweed starter cultures to farmers and the NGO's report that they have also used our suggestions in their restoration work.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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**ACRSP SUPPORT FOR INTERNATIONAL INSTITUTE FOR FISHERIES ECONOMICS AND TRADE MEETING, PORTMOUTH 2006**

*Twelfth Work Plan, Applied Technologies and Extension Methodologies 15 (12ATE15)  
Final Report*

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## **ABSTRACT**

The USAID-funded Aquaculture Research Collaborative Support Program provided financial support to the 2006 biennial conference of the International Institute of Fisheries Economics and Trade, over the period July 1, 2005 through July 31, 2006. The primary goals of this activity were to strengthen the IIFET network's capacities in aquaculture economics and seafood marketing, enabling the development of improved international research relationships, to build participation both at the conference and beyond in the area of aquaculture economics and social science development, and to improve recognition of the significance of the role of aquaculture in meeting world demand for fish products. To accomplish these goals, activities under the MOU included offering a variety of awards designed to enable and encourage participants, especially those from developing countries, to better participate in the conference, to share their research and benefit from exposure to the research of others. Two types of awards were offered: pre-conference professional awards, and best student paper awards. Under the former category, participants were selected based on their submission of an abstract describing their presentation on a relevant aquaculture-related topic; selection was carried out by IIFET Executive Director Ann Shriver in consultation with the Conference Scientific Committee. Of the selected participants, four received assistance and were able to attend the conference.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## EVALUATION AND IMPROVEMENT OF TILAPIA FINGERLING PRODUCTION AND AVAILABILITY IN HONDURAS

*Twelfth Work Plan, Seedstock Development and Availability 1 (12SDA1)  
Abstract*

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### ABSTRACT

The lack of an adequate supply of all-male tilapia fingerlings has been identified by fish farmers as a principal constraint to small- and medium-scale fish culture development in Honduras. A survey of tilapia fingerling producers was conducted to evaluate fingerling production and examine the factors that influence the way farmers produce and distribute them. Sixteen farmers were identified and interviewed during the period from September 2003 to July 2004. Seed production is concentrated in valley areas of Olancho, Comayagua, and Cortez. Fingerling sex reversal with hormone-treated feed was practiced by 14 of the 16 farmers. Seven fingerling farms are family-owned, four are private companies, one is a cooperative, one is operated by a non-profit organization, another is run by a university, and two are government stations. From each of the farms and in the manner that would be used by a typical producer, a minimum of 1,000 fingerlings were purchased and transported to the aquaculture station at Zamorano for evaluation (count, uniformity of size, and uniformity of color). A sub-sample of 250 fingerlings purchased from each farm was reared to a size when sex identification was possible. The sex of each adult fish was determined by visual examination of the genital papilla to ascertain the percent of males in each sub-sample. In aggregate, the sample produces approximately 15.3 million fingerlings a year. Most (75 %) of the fingerling producers interviewed also raise tilapia, produce other aquaculture species, and have other farm enterprises. Fingerling farmers have at least four to six years of formal education and fingerling production experience on average of 6.7 years with a range 0 to 25. This study considered three indicators of fingerling quality (uniformity of color, size and male gender). The results show that there is higher variability for color and gender than for size among the fingerling batches evaluated. This variability suggests that the quality of fingerlings delivered to tilapia farmers is not consistent. Most of the fingerling batches evaluated fall under the 90% level of uniformity of size, color, and gender. Only two independent variables had a significant relationship with fingerling quality. Farmer experience growing tilapia is positively related to fingerling quality production, but production training in itself was not related to fingerling quality as producing seed is a specialized and skilled activity. High variability in sex-reversal occurs in part because most farmers do not use standard methods of grading their fry and fingerlings by size, thus introducing inconsistency in hormone dosage and length of treatment. This is an area where training can accomplish improvement in the outcomes on the sex reversal practices as well the size uniformity of fingerlings sold. Feeding methods could be one source of low quality. Producers often do not count fry in the sex reversal process, thus feed they provide is often not well-gauged to the number of fish. Some reported that when the demand is high, they sometimes sell fingerlings before the recommended treatment period (28 to 30 days) is completed. Even though most farmers used the recommended protocol for the preparation of the hormone-treated feed (60 mg MT/kg of feed), some economize by lowering the dosage or using cheaper alcohol of a different type. Some use outdated hormone (more than four years old). One approach that has proven effective for some fingerling producers is to purchase prepared hormone feed from other farmers or institutions with more experience and access to the hormone source. Improving the level of practice among fingerling producers is a key step to improving quality and productivity in the industry.





# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## STUDIES ON STRATEGIES FOR INCREASING THE GROWTH AND SURVIVAL OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) JUVENILES REARED FOR STOCKING OR FOR USE AS BAIT

*Twelfth Work Plan, Seedstock Development and Availability 2 (12SDA2)*  
Abstract

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### ABSTRACT

The African catfish, *Clarias gariepinus*, is endemic to Kenya. It is considered to have excellent flavor and is therefore popular as a food fish. With a growing interest in aquaculture, some fish farmers are turning to the production of catfish fingerlings to sell for stocking in earthen ponds as well as for baitfish in the Lake Victoria Nile perch long-line fishery. Although spawning of *Clarias* is not a major problem, sufficient quantities of fingerlings are not being produced, due to low and highly variable rates of survival. Survival rates range from 1 to 50% in ponds, with a rate of 25% (egg to 5-gram fingerling) considered good. For producers to meet the increasing demand for fingerlings, however, techniques must be found to significantly improve these survival rates.

The primary objective of these studies has been to assess management strategies that might contribute to improved growth and survival of juvenile African catfish. Two studies were conducted by graduate students (M.Sc. candidates) at Moi University, Eldoret, Kenya, in 2005 and 2006. In one study, catfish larvae were stocked into 18 30-L glass aquaria in the hatchery, where they were offered three diet sequences and reared under two light regimes for a period of 30 days. The diet sequences tested were an *Artemia*-chick mash sequence, a rotifer-chick mash sequence, and chick mash only. Nine aquaria were illuminated and nine were darkened. Offering live feeds (*Artemia* or rotifers) prior to switching to a prepared feed (chick mash) led to better growth and survival than rearing larvae on the prepared feed only. Larvae reared in darkness had better growth and survival rates than those reared in illuminated aquaria.

The second study consisted of two separate experiments. In the first experiment, catfish larvae were reared in the hatchery for periods of 1, 5, 10, and 15 days prior to being stocked into hapas in ponds, where they were reared up to a total of 60 days. Larvae reared for 10 days prior to the transfer showed the best growth and the second best overall survival. For the second experiment, all larvae were reared in the hatchery for 10 days and then transferred to hapas, where they were stocked at densities of 25, 50, 100, and 200 fish per m<sup>2</sup> and reared for 42 days. In this experiment, stocking fish at 25 per m<sup>2</sup> resulted in the most growth and the best survival among the treatments.

All fieldwork and statistical analysis has been completed and the theses are being written. Master's theses are expected to be complete and submitted to the Graduate Committee in the School of Natural Resource Management for approval by the end of September. A final report detailing this experimental work will be completed by 30 April 2007.

The findings of this research will be applied to *C. gariepinus* fingerling production on government and private farms in Kenya. They will also be included in a new fish farming handbook being prepared under ACRSP sponsorship, providing farmers and extension workers with access to the latest information. Application of new techniques will ultimately result in increased supplies of *Clarias* fingerlings, and resulting increases in aquaculture and fishery production will contribute to human health and welfare in the region.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## CONTINUATION OF A SELECTIVE BREEDING PROGRAM FOR NILE TILAPIA TO PROVIDE QUALITY BROODSTOCK FOR CENTRAL AMERICA

*Twelfth Work Plan Seedstock Development and Availability 3 (12SDA3)  
Abstract*

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### ABSTRACT

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. The selective breeding program supported by the ACRSP 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 a wild population. 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 ACRSP project and another project supported by the National Council for Science and Technology (CONACyT-Mexico). This 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 third generation (F3) based on a combination of length and condition factor and we are currently raising a fourth generation. In this study we evaluated six tilapia lines (three more than originally proposed) in terms of growth, condition factor, fillet production, and feed conversion factor. The lines evaluated were: Tabasco-1, Control, Teapa, Wild-1, Wild-2, and Stirling. Fish were stocked in 2 m<sup>3</sup> hapas at a density of 25 fish/ m<sup>3</sup>. All hapas were placed in a single earthen pond. Average initial weight was 50 g. Best values obtained for weight corresponded to the line Tabasco-1 averaging 446.2 g at the end of the grow-out trial. This line had a 1.77 g/day growth rate. Followed by the Stirling line with an average final weight of 439.47 g (1.74 g/day). The lowest value was obtained from fish of the Teapa line (original broodstock of the state hatchery) with an average final weight of 343.86 g (1.30 g/day). Fillet yield was higher for the Tabasco-1 line (31.44% of body weight) and an average fillet weight of 130.1g, followed by the Stirling line (31.30% and 125.3 g). Once again, the lowest value corresponded to the Teapa line (28.70% and 99.6 g). From the first generation, the Tabasco-1 line has demonstrated the productive potential that is available to farmers, allowing high yields at harvest.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## DEVELOPMENT OF AQUACULTURE TECHNIQUES FOR THE INDIGENOUS SPECIES OF SOUTHERN MEXICO, *CENTROPOMUS UNDECIMALIS*: SEX DETERMINATION AND DIFFERENTIATION AND EFFECTS OF TEMPERATURE

*Twelfth Work Plan, Seedstock Development and Availability 4 (12SDA4)*  
Abstract

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### ABSTRACT

Species of “robalo,” or snook, constitute one of the most important commercial fisheries in Mexico’s gulf coast. The common snook has perhaps received the most attention and fishing pressure. On the southwestern side of the Gulf of Mexico, spawning grounds for common snook have been reported as far north as southern Texas. The Texas snook population once supported a commercial and recreational fishery, but it is now only able to support a strictly regulated recreational fishery. In Mexico, there is a trend for diminishing catch volumes for common snook, a situation that has led to concerns for the regional snook fisheries and to calls for improved management practices. Thus, the development of culture techniques for common snook would benefit a common resource in the US and Mexico by providing relief from fishing pressure on wild snook stocks.

Female common snook are larger than males of the same age class, especially in younger fish. Thus, female snook may have an intrinsically faster growth rate than males. The present study focuses on an evaluation of rearing techniques to skew sex ratios toward females and lead to enhanced growth rates for farmed common snook. In order to accomplish this objective, it is first necessary to establish the pattern and timing of gonadal sex differentiation. Although it has been reported that common snook are protandric — they first develop as males before changing sex into females — basic information about gonadal sex differentiation is not available for this species. This information is needed to determine the time at which treatment can be applied to feminize snook fry and bypass the male phase.

In Mexico, 256 juvenile snook were collected. Otoliths from 228 fish were extracted and processed. Results indicate that age can be determined up to day 100. After this point, the daily marks get too close to be used accurately. The histological analyses of gonads indicate that testicular differentiation initiates between 91 and 123 days of age (fork length ranging between 11 and 21.1 cm). At this stage, spermatogonia are clearly identified and efferent ducts are present. All fish analyzed differentiated as males and no indication of female development was observed. This information suggests that treatment for sex inversion needs to begin between 60 and 70 days of age (3–6 cm). During June–July 2006, 30 adult snook were captured alive in Tabasco (400–6000 g; 30–95 cm) to produce fry for experimentation; however, only six have survived (2 females and 4 males). More captures are scheduled to ensure enough fish to induce spawning. In addition, a recirculating system containing 21 70 L fiberglass tanks for fry rearing has been built and tested. Our feminization experiments will be conducted between August and December 2006.

In Texas, the lower portion of the Rio Grande is believed to be important nursery habitat for juvenile snook. However, little knowledge is available about the riverine habitat requirements for juvenile snook and its early reproductive development. The objectives of the US component of the study are to examine the pattern and timing of the early gonadal development of Texas snook populations and to compare with observations for common snook in Mexico (Tabasco). Snook were sampled from January through March 2006 along the lower 51.5 km of the river. We captured 225 common snook, all above river kilometer 12.9. All fish <13.4 cm contained indifferent gonads and are believed to be young-of-the-year individuals; larger fish, up to 67.4

cm, were males (including juveniles and adults) except for one female (61.4 cm). Fish age will be confirmed by counting annuli on otoliths. The results of this study will provide useful information to understand the reproductive biology of the snook populations inhabiting the Texas-Mexico gulf coast. In addition, these results are expected to assist in efforts to manage the Texas snook population. The US component of the study also aims to characterize habitat requirements for juvenile snook and is being supplemented by funds or in-kind support from the US Geological Survey, Texas Tech University, and Texas Parks and Wildlife.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## KENYA CAPACITY BUILDING: STUDENT RESEARCH AND THESIS SUPPORT

*Twelfth Work Plan, Seedstock Development and Availability 5 (12SDA5)  
Abstract*

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### ABSTRACT

This activity addresses new objectives for the OSU/Kenya Project for the 2005–2006 year. During this year we will provide scholarship support for the research and thesis work of two new graduate students currently pursuing aquaculture studies at Moi University, Eldoret, Kenya. We will also provide stipend support for three undergraduate aquaculture students working on senior projects. This work may be conducted either at Moi University, Eldoret, or Sagana Fish Farm, Sagana. Support is also provided for one of our current graduate students to present research results at the “AQUA 2006” conference in Florence, Italy.

Research topics being undertaken by the new students include work on 1) the fecundity and energetics of tilapia (*Oreochromis niloticus*) brooders conditioned under different feeding regimes and 2) yields of Nile tilapia and African catfish (*Clarias gariepinus*) reared together in different stocking ratios. It is expected that they will complete their fieldwork on these topics by September 2006 and finish their theses by late spring 2007.

Current graduate student Victoria Boit traveled to Florence, Italy, to present the results of her research in the CRSP session of AQUA 2006 on Saturday, May 13, 2006. Her presentation was entitled “Effects of three feeding regimes and two light regimes on the growth and survival of African catfish *Clarias gariepinus* fry in aquaria.” Victoria is in the final stages of preparing her thesis and is expecting to submit it to her committee by September 2006.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## EFFECTS OF NATIVE PERUVIAN FEEDSTUFFS ON GROWTH AND HEALTH OF *COLOSSOMA* AND *PIARACTUS*

*Twelfth Work Plan, Fish Nutrition and Feed Technology 1 (12FNF1)*  
Abstract

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### ABSTRACT

A feeding trial with juvenile (22.5 g) *Colossoma macropomum* was conducted at the University of Arkansas at Pine Bluff to determine the effects of cooked or uncooked plant feedstuffs (plantain, pijuayo, and yucca) on fish growth, survival, and health. These high-carbohydrate feedstuffs are readily available in Peru and have the potential to replace more expensive or less available ingredients in Characid diets. The control diet was similar to a commercial formulation for channel catfish (containing fish meal, soybean meal, wheat, corn, rice bran, soybean oil, and supplemental vitamins and minerals). The experimental feedstuffs replaced wheat in the control diet. Starch was the primary carbohydrate in wheat and all of the test feedstuffs. The available energy from cooked starch is reportedly higher than that from uncooked starch in some fish species, so we tested both versions of each feedstuff within the same trial. Diets were similar in total protein (33–34%) and other ingredients except for the experimental feedstuffs (inclusion rate: 30%). Growth rates were high, and three fish were removed from each tank six weeks into the study to allow the remaining fish to resume rapid growth. Liver glycogen of the fish that were removed from the study did not differ due to dietary treatment. After 12 weeks, there were no differences in growth or survival of fish due to diet. Lysozyme activity was not detected, but it is possible that the pH used in our standard protocol is not appropriate for this species. Additional work is needed to determine the optimal pH for lysozyme assays for *C. macropomum* (and for *P. brachyponus*, which also had no detectable lysozyme activity in a previous trial). Based on growth, survival, and liver glycogen, these feedstuffs are all suitable, practical carbohydrate sources for *C. macropomum*. Furthermore, there was no indication that cooking increased the available energy of plantain, pijuayo, or yucca for this species.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## NUTRITION AND NUTRIENT UTILIZATION IN NATIVE PERUVIAN FISHES

*Twelfth Work Plan, Fish Nutrition and Feed Technology 2(12FNF2)  
Final Report*

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### ABSTRACT

*Colossoma macropomum* (Characiformes: Characidae) is the second-largest scaled, freshwater fish in South America. *C. macropomum* (black-finned pacu) is native to the Amazon Basin and possesses many characteristics suitable for aquaculture. Black-finned pacu is in high demand and brings a high price at the marketplace. However, no formulated diets are available specifically for *C. macropomum* culture. Consequently, a wide range of ingredients for locally manufactured formulated diets is used in the countries where this fish is cultured. These diets have variable crude protein (CP) ranging from 18 to 43%, and the supplied ration ranges from 1 to 5% of the fish's wet body weight. Commonly, wheat, corn, and rice are one of the main energy sources in these formulated diets. As wheat is not traditionally cultured in the Amazonian region, it has to be imported from distant regions, thus limiting its use for direct human consumption. A growth experiment was conducted to determine the effect of substituting three alternative ingredients for wheat middlings on growth performance and conversion efficiency of *C. macropomum*. Fish ( $86.9 \pm 6.4$  g) were fed four practical diets: 1) control diet (31.8% CP), 2) yucca *Manihot sculenta* diet (27% CP), 3) plantain *Manihot sculenta* diet (27.5% CP), and 4) pijuayo *Bactris gasipaes* (palm peach) diet (28.1%) for a 24-wk period and their weight (g), length (cm), specific growth rates (SGR), food conversion ratios (FCR), and protein efficiency ratio (PER) were determined and compared. Fish were fed 3% of BWD divided in two daily rations. Weight and length were measured every two weeks to adjust their feed allotments. Final mean weights for *C. macropomum* fed the control, yucca, plantain, and pijuayo (palm peach) diets were 538.8, 559.0, 552.7, and 527.4 g, respectively, and were not significantly different ( $P>0.05$ ). Final mean weight gained of *C. macropomum* fed the control, yucca, plantain, and pijuayo diets were 458.2, 476.2, 465.8, and 437.8 g, respectively, and were also not significantly different ( $P>0.05$ ). SGR and FCR were not significantly influenced by dietary treatments ( $P>0.05$ ); however, PER was significantly affected ( $P<0.05$ ). Based on these findings, it was concluded that any of the tested ingredients (yucca, plantain, or pijuayo meal) might serve as carbohydrate sources in formulated diets for *C. macropomum* without negatively influencing fish growth performance.

In a second experiment, the digestible energy and apparent nutrient digestibility coefficients of three plant-source ingredients common to the Amazon Basin (pijuayo, plantain, and yucca) were determined for black pacu, *Colossoma macropomum*, averaging  $61.05 \pm 16.96$  g. Fish were fed pelleted practical diets to apparent satiation and the feces were collected in specially designed chambers. The digestibility value for each ingredient was determined by comparison to the digestibility of the test diet to a reference diet (27.6% crude protein and 1% chromic oxide). The digestible energy values of pijuayo (PI), plantain (PL), and yucca (YU) were 4,518, 4,386, and 4,355 kcal kg<sup>-1</sup>, respectively, while the apparent dry matter digestibility coefficients were 91.7, 87.6, and 65.8%, respectively. The apparent crude protein digestibility coefficients were 81.0, 32.9 and 30.1% for PI, PL, and YU, respectively, and the apparent lipid digestibility coefficients were 89.0, 77.9, and 41.2%, respectively. These results suggest that of the three plant sources tested only pijuayo can likely be utilized as an ingredient in commercial pelleted diets for black pacu without compromising the assimilation of dietary intake of protein, lipids or dry matter.

## INTRODUCTION

The need exists to further evaluate the aquaculture potential of local and native species and to develop appropriate culture technologies. The frugivorous fish *Colossoma macropomum* (commonly called black pacu in English, gamitana in Peru, cachama negra in Colombia and Ecuador, cachama in Venezuela, and tambaqui in Brazil) and *Piaractus brachypomus* (commonly called red pacu in English, paco in Peru, cachama blanca in Colombia and Ecuador, morocoto in Venezuela and pirapitinga in Brazil) are native to the Amazon Basin. They as well as their hybrids possess many characteristics suitable for aquaculture. 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 IAP for many years (Alcantara; IAP; personal communication), as well as in Brazil (Carneiro, 1981; Hernandez et al., 1992). The diets of wild *C. macropomum* are about 20-30% protein, with 75% of the protein being of plant origin (Araujo-Lima and Goulding, 1997). Fish diets 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 (Kubitzi 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.

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., 2004). 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. In Workplan 11, digestibility trials were conducted with *P. brachypomus* utilizing three native feedstuffs (plantain *Musa paradisiacal*, yucca *Manihot sculenta*, and pijuayo *Bactris gasipaes*) in 110-L tanks

in a flow-through system. Digestible energy, protein, lipid and dry matter digestibility coefficients were determined for each feedstuff. The reference diet was similar in composition to those used currently for Characid fishes at IAP (Peru). Digestibility coefficients were determined by using an indirect method, involving chromic oxide ( $Cr_2O_3$ ) as a non-digestible marker. The digestibility of crude proteins (85.6%), crude fat (90.4%), and energy (70.3%) of pijuayo in *P. brachypomus* was far superior to that of yucca and plantain. The digestibility of plantain and yucca by *P. brachypomus* were very similar to each other for crude proteins (57.5 vs. 53.0%), crude fat (54.9 vs. 64.8%), and energy (29.0 vs. 21.0%). Pijuayo appears to be an excellent ingredient for use in formulated diets for *P. brachypomus*. Additionally, the abundance of pijuayo in the Amazon Basin makes this fruit economically viable to the small-scale farmers to reduce feed manufacturing cost. Identical studies were conducted in Workplan 11.5 with *Colossoma macropomum* as well as a grow-out trial utilizing diets containing native plants as ingredients.

## MATERIALS AND METHODS

### Objective 1. Assess the feasibility of utilizing native Amazonian plant products for small-scale sustainable aquaculture production of gamitana (*Colossoma macropomum*).

A growth experiment was conducted to determine the effect of substituting three alternative ingredients for wheat middlings on growth performance and conversion efficiency of *C. macropomum*. Fish ( $86.9 \pm 6.4$  g) were fed four practical diets: 1) control diet (31.8% CP), 2) yucca *Manihot sculenta* diet (27% CP), 3) plantain *Manihot sculenta* diet (27.5% CP), and 4) pijuayo *Bactris gasipaes* (palm peach) diet (28.1%) for a 24-wk period. Fish were fed 3% of BWD divided in two daily rations. Weight and length were measured every two weeks to adjust their feed allotments. Weight (g), length (cm), specific growth rates (SGR), food conversion ratio (FCR), and protein efficiency ratio (PER) were determined and compared.

The feeding trial was conducted in a 28,000 L recirculating system. Flow rate was regulated to obtain a complete water exchange for each tank per day. Temperature and dissolved oxygen were measured twice per day before feeding. Total ammonia nitrogen, nitrite, alkalinity and pH levels were measured twice per week.

The experiment involved four diets (Table 1) evaluated in triplicate. Diets were prepared in a mixer by slowly adding micro-nutrients (vitamin and minerals premixes) to the macro-ingredients to ensure a homogenous mixture. About 400-450 mL of water was added per kilogram of diet to achieve a consistency that would produce stable pellets. A meat grinder fitted with a 3 mm die was used to produce the pellets, which were fan dried for 24 h and stored at  $-18^\circ C$  until used. Moisture and dry matter content of the experimental diets were determined by drying triplicated samples weighing 250 mg at  $135^\circ C$  for 3 h in an oven. Samples were weighed on an analytical scale. Crude protein was determined using a distillation unit. A 1 g sample was weighed on nitrogen-free paper and transferred to a digestion tube along with two Kjeldahl catalyst tablets. Concentrated sulfuric acid was added using a dispenser and the tubes were placed in a preheated digestion unit at  $420^\circ C$  for 1 h. After cooling, the tubes were distilled automatically in the distillation unit. The solution in the receiver flask was



Table 1. Composition of the reference and experimental diets (%) used to feed gamitana (*Colossoma macropomum*) juveniles in a 24-wk feeding trial

Ingredients (%)	Control	Cassava	Pijuayo	Plantain
Menhaden fish meal	9.0	9.0	9.0	9.0
Soybean meal	35.0	35.0	35.0	35.0
Corn grains	9.8	9.8	9.8	9.8
Wheat middlings	30.0	0.0	0.0	0.0
Rice Bran	12.0	12.0	12.0	12.0
Soybean oil	3.0	3.0	3.0	3.0
Trout Vitamin Premix	0.5	0.5	0.5	0.5
Trout Mineral Premix	0.5	0.5	0.5	0.5
Vitamin C (Stay C 35%)	0.2	0.2	0.2	0.2
Test ingredient	0.0	30.0	30.0	30.0
Dry Matter	89.7	89.4	89.4	87.6
Crude Protein	31.8	27.5	27.0	28.1
Crude Fat	6.5	5.3	5.1	9.7
Total Ash	7.9	6.4	6.9	6.8
Digestible Energy (kcal/kg)	2,540	2,930	2,935	2,938

then titrated against HCl of a known concentration. Percent nitrogen of the sample was calculated using the titration volume and converted to protein using a factor of 6.25. Crude fat was determined by the lipid extraction method according to Folch (1957). Ash was obtained by burning 2 g of diet samples at 550 °C for 3 h in a muffle furnace. Energy content was obtained using a bomb calorimeter.

Hatchery-produced *C. macropomum* juveniles (86.9 ± 6.4 g) were utilized in the experimental trials. Each treatment (diet), including the control diet, had three replicates with 28 fish per replicate. Uniform-sized fish were selected from a large population, weighed and randomly assigned to experimental circular tanks. Fish were acclimated to the experimental system and their respective diets for 7-d before starting the experiment. Fish were fed 3% of their body biomass divided in two daily rations, seven days a week. Feed rations for each replicate were adjusted every two weeks by estimating the fish total biomass in the tanks by sub-sampling each replicate, respectively. In each sub-sampling, fish weight was recorded to the nearest 0.1 g. At the end of the experiment, the following variables were calculated:

$$\text{Specific growth rates (SGR)} = \frac{(\ln \text{ final body weight} - \ln \text{ initial body weight}) \times 100}{t \text{ (days)}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g) dry basis}}{\text{wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{wet weight gain (g)}}{\text{protein intake (g) dry basis}}$$

Data obtained in the experiment were analyzed by JMP statistical software using one-way ANOVA and expressed as the mean ± standard error (SEM). Multiple comparisons of means were performed using Tukey's HSD test. The level of significance was  $P < 0.05$ .

**Objective 2. Compare nutrient digestibility of endemic Peruvian plant products in gamitana (*Colossoma macropomum*) with that of feedstuffs currently used in Characid diets.**

This experiment was conducted to determine differences in digestibility of three native food sources (plantain *Musa paradisiaca*, yucca *Manihot sculenta*, and pijuayo *Bactris gasipaes*) commonly fed to fish species cultured in the Peruvian Amazon. The three ingredients (Table 1) were tested with *C. macropomum* using a catfish reference diet (32% protein) as a control. As very few feedstuffs are the sole component of a fish diet, digestibility was studied in combination with other ingredients in the assay diets. The reference diet utilized menhaden fish meal because of the difficulty in acquiring anchovy fish meal in the US.

Digestibility trials were conducted in 110-L tanks in a flow-through system at SIUC using the indirect method with chromic oxide (1%) as the marker (Reilly and Lochmann, 2000). The assumption was that the amount of the marker in the feed and feces remains constant throughout the experimental period and that all of the ingested marker will appear in the feces. Digestible energy, protein, lipid and dry matter digestibility coefficients were determined for each feedstuff. The reference diet was similar in composition to those currently used for Characid fishes in Peru. Water quality was maintained by a flow rate of 36 L/min and a biofilter and bead filter. Temperature was held at 27 ± 2 °C.

Five *C. macropomum* (~80 g) were placed in each of nine randomly distributed 110-L tanks, each containing a digestibility chamber. Fish were fed for a 6-d acclimation period followed by a 7-d fecal collection period. During the fecal collection period fish were fed to apparent satiation once daily at 18:00 h and their feces collected 12 to 16 h post-prandial. Fecal samples were stored frozen in airtight plastic bags. Feed and feces were analyzed for dry matter (135 °C for 3 h), gross energy content (using an adiabatic bomb calorimeter), protein (Kjeldahl method – AOAC, 1984) and total lipid (using the modified lipid extraction according to Folch 1957). Chromic oxide levels were analyzed by AOAC methods (1990). The digestibility of the nutrients were determined by assessing the difference between the feed and fecal concentrations of the marker and

the nutrient or energy. The percent nutrient digestibility was estimated using the following formula:

$$\text{Nutrient Digestion Coefficient} = \frac{100 - (100 \% \text{ marker in the feed}) (\% \text{ nutrient in feces})}{(\% \text{ marker in feces}) (\% \text{ marker in feed})}$$

The apparent digestibility coefficient (ADC) for a nutrient in each test ingredient was calculated using the following expression:

$$\text{ADC} = \frac{100}{30 \times \text{dig. coeff. of test diet}} - \frac{70}{100 \times \text{dig. coeff. of reference diet}}$$

**Statistical Analysis**

Data on chromic oxide recovery was analyzed using One-way ANOVA and presented as means (± SD) of three replicates, followed by Tukey least significant difference test when the ANOVA indicated differences in treatment means (P≤0.05) existed.

*Null Hypothesis for grow-out trials:* There is no difference on growth performance in *C. macropomum* fed diets containing different native plants.

*Null Hypothesis for Digestibility trials:* There is no difference in digestibility of energy, dry matter, protein or lipid between feedstuffs for *C. macropomum*.

**RESULTS**

**Objective 1. Assess the feasibility of utilizing native Amazonian plant products for small-scale sustainable aquaculture production of gamitana (*Colossoma macropomum*).**

Yucca, palm peach, and plantain proved to be equally effective as replacements for wheat middlings at 30% in black pacu diets. The final mean body weights of the fish were 526.8, 535.3, 558.2 and 525.9 g for control, yucca, plantain and pijuayo diets, respectively, and were not significantly different (P>0.05). Final weight gain for control, yucca, peach palm, and plantain diets were 458.2, 476.2, 437.8, and 465.8 g, respectively, and were not significantly different (P>0.05), nor were final body weights gains (%), SGR and FCR. Black pacu fed plantain, yucca, and peach palm had a significant higher PER values (Table

2) than black pacu fed the control diet (P<0.05). Further, black pacu on the plantain diet had the highest PER values (1.83 ± 0.08) among all treatments tested.

Mean values for water quality parameters (DO= 5.59 ± 0.47 mg/L, temperature= 27.3 ± 1.1 °C, pH= 6.8 ± 0.1, ammonia = 0.26 ± 0.1 mg/L, nitrite = 0.05 ± 0.01 mg/L, and alkalinity 50.4 ± 9.8 mg/L) recorded during the 24-wk experiment were adequate for black pacu culture (Castagnolli 2000; Fernandes et al. 2001).

**Objective 2. Compare nutrient digestibility of endemic Peruvian plant products in gamitana (*Colossoma macropomum*) with that of feedstuffs currently used in Characid diets.**

The digestible energy and apparent nutrient digestibility coefficients of three plant-source ingredients (Table 3) common to the Amazon Basin, pijuayo, plantain, and yucca, were determined for *C. macropomum* (61.05 ± 16.96 g initial weight). Fish were fed pelleted practical diets to apparent satiation and the feces were collected in specially designed chambers. The digestibility value for each ingredient was determined by comparison to the digestibility of the test diet to a reference diet (27.6% crude protein and 1% chromic oxide). The digestible energy values (Table 3) of pijuayo (PI), plantain (PL), and yucca (YU) were 4,518, 4,386, and 4,355 kcal/kg, respectively, while the apparent dry matter digestibility coefficients were 91.7, 87.6, and 65.8%, respectively. The apparent crude protein digestibility coefficients were 81.0, 32.9 and 30.1% for PI, PL, and YU, respectively, and the apparent lipid digestibility coefficients were 89.0, 77.9, and 41.2%, respectively.

Table 3. Apparent digestibility coefficients (%) of major nutrients in the three experimental feed ingredients fed to black pacu *Colossoma macropomum*.

Ingredient	Protein (%)	Lipids (%)	Org Matter (%)	Energy (kcal/kg)
Pijuayo (whole)	81.0 ±	89.0 ±	91.7 ±	4,518
Plantain w/o rind	32.9 ±	77.9 ±	87.6 ±	4,386
Yucca w/o rind	30.1 ±	41.2 ±	65.8 ±	4,355

\* Values within each column having different superscript letters are significantly different (P<0.05)

Table 2. Growth performance and feed utilization of *Colossoma macropomum* juveniles fed a reference and three experimental diets for 24-wk (mean values ± SEM). Mean values sharing the same superscript are not significantly different (P> 0.05). IBW (initial body weight), FBW (final body weight), BWG (body weight gain), SGR (specific growth rate), FCR (feed conversion ratio), and PER (protein efficiency ratio).

Treatments	Control	Yucca	Pijuayo	Plantain	P Value
IBW (g)	80.6 ± 2.2 <sup>a</sup>	82.8 ± 4.0 <sup>a</sup>	89.6 ± 7.2 <sup>a</sup>	86.9 ± 4.1 <sup>a</sup>	0.5678
FBW (g)	538.8 ± 12.4 <sup>a</sup>	559.0 ± 23.1 <sup>a</sup>	527.4 ± 15.4 <sup>a</sup>	552.7 ± 34.8 <sup>a</sup>	0.7716
BWG (g)	458.2 ± 11.3 <sup>a</sup>	476.2 ± 22.7 <sup>a</sup>	437.8 ± 22.1 <sup>a</sup>	465.8 ± 36.9 <sup>a</sup>	0.7417
BWG (%)	669.0 ± 16.4 <sup>a</sup>	678.0 ± 39.4 <sup>a</sup>	599.0 ± 64.1 <sup>a</sup>	640.6 ± 58.2 <sup>a</sup>	0.6667
SGR	1.02 ± 0.01 <sup>a</sup>	1.03 ± 0.03 <sup>a</sup>	0.95 ± 0.06 <sup>a</sup>	0.99 ± 0.05 <sup>a</sup>	0.6242
FCR	1.98 ± 0.04 <sup>a</sup>	1.86 ± 0.08 <sup>a</sup>	1.84 ± 0.09 <sup>a</sup>	1.84 ± 0.01 <sup>a</sup>	0.3746
PER	1.42 ± 0.03 <sup>a</sup>	1.76 ± 0.07 <sup>b</sup>	1.78 ± 0.02 <sup>b</sup>	1.83 ± 0.08 <sup>b</sup>	0.0042

## DISCUSSION

**Objective 1. Assess the feasibility of utilizing native Amazonian plant products for small-scale sustainable aquaculture production of gamitana (*Colossoma macropomum*).**

Final individual body weights of the fish obtained in the grow-out study might be considered satisfactory for *C. macropomum* since under normal pond culture conditions this fish is reported to reach between 0.8 kg and 1.2 kg in one year, depending on the feed quality and quantity (Alcántara et al., 2001). Several grow-out studies have been performed in ponds testing different protein levels and sources, and stocking rates with different results. For instance, Roubach and Saint-Paul (1993) determined that *Colossoma* fingerlings fed a control diet containing 25% fishmeal, 30% soybean meal, 23% corn, and 20% wheat (35% crude protein content) had a better growth performance than those fed munguba *Pseudobombax munguba* seeds, wild rice *Oryza* spp., rubber-tree *Hevea brasiliensis* seeds and *Cecropia latiloba* fruits, which had lower protein levels.

Daily fish growth rates obtained in the study were in the order of 1.02, 1.03, 0.95, and 0.99 % per day for the control, yucca, pijuayo and plantain diets, respectively, and are similar to those obtained by other researchers. Roubach and Saint-Paul (1993) obtained daily fish growth values of 0.80, 0.98, 1.26, and 2.53 % per day for *Colossoma* juveniles (~18 g) fed rubber-tree *Hevea* spp. seeds, wild-rice *Oryza* spp., munguba *Pseudobombax munguba* seeds, and a control diet (35% CP content). In a feeding trial testing two diets containing 27.5 and 42.1% of crude protein (Saint Paul 1986), *Colossoma* juveniles grew at a daily rate of 0.8 and 0.9 %, respectively. While feeding *Colossoma* juveniles with biological ensilage in the Brazilian Amazon, Ximenes-Carneiro (1991) obtained daily growth rates of 0.52% and 0.53%, which are far lower than the specific growth rates obtained in this study.

Feed conversion ratios (FCR) obtained in our study are acceptable considering FCR values recorded by Roubach and Saint-Paul (1993) ranged from as low as 1.8 (control) to as high as 8.9 (*Hevea* spp. seeds). Protein efficiency ratio (PER) values ranged from 1.6 (control) to 3.9 (*Oryza* sp.). Taking into account the protein content of the control diet and the fruits, a significant increase in protein content resulted in better growth rates and feed conversion.

Since production of plantain and yucca are high in Latin America (FAO, 1990) and pijuayo is also very abundant in most of the Amazonian region, the utilization of these ingredients in formulated diets for fish farming might be an excellent alternative to reduce the feeding expenditures and overall production costs replacing wheat derivatives. Traditionally, the use of wheat in pacu's rations ranges from as low as 5% to as high as 35% (TCA 1993). We used wheat at a 30% level with encouraging results. Mori et al. (1999) recorded no significant differences on growth of *C. macropomum* fingerlings when utilizing pijuayo meal instead of corn, suggesting pijuayo meal can easily replace corn in balanced diets for this species.

## CONCLUSIONS

The experimental diets tested in the grow-out experiment performed equal or slightly better than the control diet. This is an important outcome suggesting either yucca, plantain,

or pijuayo meal might effectively replace wheat in *C. macropomum* diets in South America without negatively affecting fish growth rates. Likewise, feed conversion ratios were not adversely affected. These feedstuffs offer potential as alternatives for lowering the costs of diets in the Amazon region. The results of the digestibility study suggest, however, that of the three plant sources tested only pijuayo presents greater potential to be utilized as an ingredient in commercial pelleted diets for *C. macropomum* in the Amazon basin since it does not compromise dietary protein, lipid or dry matter assimilation.

## ANTICIPATED BENEFITS

The development of sustainable aquaculture of *Colossoma* and *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 rainforest 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* spp. should relieve some of the fishing pressure on these overharvested native species. The project is providing 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 supports 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.

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## USE OF PHYTOCHEMICALS AS A NEW METHOD TO SEX-REVERSE NILE TILAPIA AND TROPICAL GARFISH. PART I: USE OF AQUEOUS PLANT EXTRACTS IN TILAPIA

*Twelfth Work Plan, Fish Nutrition and Feed Technology 3 (12FNF3a)  
Final Report*

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### ABSTRACT

Many studies have focused on the use of chemicals produced by plants (phytochemicals) that can be characterized as mimics of sex steroid endocrine regulators. It is anticipated that phytochemicals will act as endocrine modulators by changing endogenous hormone profiles. These effects could be related to their inhibitory capacity towards aromatase activity and many other unidentified mechanisms. If such activity is precisely expressed by these chemicals, they could provide a novel (alternative to synthetic inhibitors) mode of action to induce changes in the phenotypic process of sex differentiation in fish gonads, especially in tilapia. Natural chemicals, because of their expected safer utilization and handling issues, and possibly lower toxicity for both fish and the surrounding environment, are very attractive alternatives.

We have conducted a series of feeding trials on first feeding all-female Nile tilapia (>80% female). Phytochemicals have been obtained as aqueous extracts from *Hibiscus macranthus*, mate (*Ilex sp.*), and maca (*Lepidium meyenii*) by suspension of dry plant material in distilled water for 12 hours. After dissolution, the extracts were filtered using paper filters. The resulting suspended solutions were freeze-dried to obtain dry powder extracts. These extracts were added to casein-gelatin based diets at different concentrations and fed to fish. Diets free of phytochemicals and diets supplemented with two different synthetic chemicals, steroid hormones, 17 $\alpha$ -methyltestosterone (MT) and spironolactone (SPIRO) were used as positive controls in a second feeding experiment. The preliminary results indicate that the sex ratio is not affected by the inclusion of the tested plant extracts; however, MT and SPIRO fed fish exhibited significant change in sex ratio, 100% and 75% males, respectively. In addition, no significant differences were observed in the final individual body weight, specific growth rate, or food conversion ratio among dietary groups in all experiments. The search for active phytochemicals affecting gonad differentiation in fish should continue, with further purification, diversification of extraction methods, and examination of more plant species.

### INTRODUCTION

In tilapia culture, all-male populations are desirable because males demonstrate superior growth characteristics when compared to females (Mbahinzireki et al., 2001). Moreover, the culture of monosex 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 (Abucay and Mair, 1997; Gale et al., 1999; Green and Teichert-Coddington, 2000). 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). Thus, the later factors and the restricted use of MT, given its potential as environmental hazard and health risks, new alternative methods to produce all-male tilapia populations are needed and are among the priorities of the Aquaculture Collaborative Research Support

Program (ACRSP). Such alternatives include phytochemicals naturally found in numerous plants that exhibit endocrine disruptive activity since they interfere with various enzymatic reactions either in steroid metabolism (aromatization) or in the mechanism of steroid action (competitive receptor binding) (Pelissero et al., 1996; Geahlen et al., 1989). Therefore, we intended in the present work to continue the isolation and development of steroid-free methods for producing all-male populations of tilapia using either a suite of active compounds (plant extracts) or specific purified chemicals. We examined if dietary administration of purified chemicals and use of extracts from plants have some effects on hormonal regulation. It was reported that *Hibiscus macranthus* and *Basella alba* induced anabolizing and virilizing effects in rats (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 human male infertility in Peruvian rural communities (Quiros et al., 1996; Cicero et al., 2001; Gonzales et al., 2003). The 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 pulverised maca root improved sexual performance of male rats. Furthermore, aqueous extracts of maca roots administered for 14 days to rats increased the weight of testis and the size of the epididymis, and enhanced spermatogenesis (Gonzales et al., 2001).

Therefore, both pathways, and enzymatic inhibition or receptor competition (as agonist or antagonist of hormonal pathways) will be considered in our approach. In this approach, we used natural high concentrations of phytochemicals with respect to tilapia diets and determined possible synergistic effects of several phytochemicals. We hypothesized that feeding fish with diets containing these natural substances would affect the sex ratio of tilapia when exposed during undifferentiated juvenile stages. The evaluation requires inclusion of natural sources of these compounds (plant extracts) for dietary supplementation, as well as testing their efficacy in parallel to other synthetic substances (SPIRO and MT).

## MATERIALS AND METHODS

### All-female tilapia production

For this purpose we focused on the production of all-female genotype tilapia progenies in our laboratory. Fish originally obtained as genetically all-female tilapia *Oreochromis niloticus* from Phil-FishGen, Nueva Ecija, Philippines, had some individual males in the stock. Thus, 50 fish were identified, tagged with PITs (passive integrated transponders; Destron Fearing Co., St Pail, MN), and separated into both genders (45 females and 5 males). Parental groups of a male:female 1:5 ratio from the identified fish were sorted into individual tanks and checked biweekly to remove any fertilized eggs, embryos or hatched larvae from the mouths of females. Progenies were reared separately until they reached a minimum weight of 1.5 g. We produced 12 progenies from tagged fish with different sex ratios in their progenies.

### Feeding trials

We conducted two feeding trials using the first feeding all-female Nile tilapia (>80% female) pooled from the spawns of 2-3 previously identified females with a specific male (#1 in Fig. 1). In both feeding experiments, once larvae were produced, fish were randomly distributed into glass aquaria in a recirculation system at a temperature  $26 \pm 2$  °C, at a density of 50 and 60 fish per aquarium in experiments 1 and 2, respectively, with three replicates per dietary treatment.

All plant aqueous extracts were added to the diets on a dry matter basis. To obtain the aqueous extracts, 20 g of dry plant material was suspended in 1.5 l of distilled water for 12 h, filtered using paper filters and the resulting suspended solutions were freeze-dried to obtain dry powder extracts. Powder extracts were suspended in 10 ml of dimethylsulphoxide (DMSO) prior to diet mixing in order to facilitate incorporation to the experimental diet.

To carry out feeding experiment 1, the following casein-gelatin based diets (Rodriguez-Montes, 2005) were prepared: control (CON), 0.06% 17 $\alpha$ -methyltestosterone (MT), aqueous extract of

0.01% (H10) and 0.05% (H50) of *Hibiscus spp.* seeds. Fish were fed for 42 d, and periodical weight gain was estimated at 14 and 28 d to readjust the feeding ratio from 20 to 8% body weight per day. The MT diet was withdrawn at day 28, and fish were fed with control diet for the remainder of the experiment.

To perform feeding experiment 2, casein-gelatin based diets were prepared as follows: control (CON), 0.06% 17 $\alpha$ -methyltestosterone (MT), 1% spironolactone (SPIRO), along with aqueous extracts of 0.1% (H100) and 0.5% (H500) of *Hibiscus macranthus* seeds and mate (*Ilex sp.*) leaves (M100 and M500 respectively), and 0.5% of maca roots (*Lepidium meyenii*) (MACA). Fish were fed for 40 d, and weight gains were evaluated at 14 and 28 d to readjust the feeding ratio. Again, the MT diet was withdrawn at day 28, and fish were fed the control diet (no supplement) for the remaining duration of the experiment. Sex was determined by microscopic analyses of gonad squashes (Guerrero and Shelton, 1974). At the end of experiments (40–42 days) fish were transferred to a commercial feed and maintained until the mean weight of 1–2 g, when they were sexed. In the case of individual male-females crosses, 30 progenies were examined for gonads. In the case of samples of fish from feeding experiments (30 fish per tank; total 90 fish per treatment; except SPIRO treatment where  $n=79$ ), fish were anesthetized and euthanized in ice-water slurry at the age of 55–60 days (fish were larger than listed in Table 1), and the gonads dissected manually for examination. In both trials, fish performance was evaluated in terms of the

Table 1: The observed final mean weights of tilapia, specific growth (SGR) rate and survival in experiments 1 and 2

Treatment	Experiment 1 (42 d)		
	Final mean weight (g)	Survival (%)	SGR (%/d)
CON	0.74 $\pm$ 0.02	99 $\pm$ 0	11.1 $\pm$ 0.3
MT	0.73 $\pm$ 0.03	98 $\pm$ 1	11.2 $\pm$ 0.4
H10	0.71 $\pm$ 0.01	98 $\pm$ 1	11.2 $\pm$ 0.4
H50	0.71 $\pm$ 0.01	98 $\pm$ 1	11.7 $\pm$ 0.1
Treatment	Experiment 2 (40 d)		
	Final mean weight (g)	Survival (%)	SGR (%/d)
CON	0.80 $\pm$ 0.03	98.3 $\pm$ 0.0	11.0 $\pm$ 0.1
MT	0.75 $\pm$ 0.05	90.6 $\pm$ 7.9	10.8 $\pm$ 0.2
SPIRO	0.59 $\pm$ 0.18	49.4 $\pm$ 5.8*	10.1 $\pm$ 0.8
MACA	0.74 $\pm$ 0.02	94.4 $\pm$ 5.1	10.8 $\pm$ 0.1
H100	0.75 $\pm$ 0.03	96.1 $\pm$ 2.5	10.8 $\pm$ 0.2
H500	0.65 $\pm$ 0.04	93.3 $\pm$ 8.8	10.4 $\pm$ 0.3
M100	0.75 $\pm$ 0.05	95.0 $\pm$ 1.7	10.7 $\pm$ 0.1
M500	0.64 $\pm$ 0.07	92.8 $\pm$ 3.5	10.4 $\pm$ 0.2

\*Indicates statistically significant differences in column (P<0.05).

final individual body weight (g), survival (%) and specific growth rate (SGR, %/day). The final sex was determined by microscopic analysis of gonad squashes at the end of experiment. Statistical analyses of growth data was performed by one way ANOVA and possible sex ratio differences were analyzed using contingency tables for chi-square test.

**RESULTS**

**All-female tilapia production**

The observed sex ratio in the evaluated progenies depended on the parental couple. The sex ratio varied for a single male from 38 to 100% females, and at least 4 progenies were found with 70–85% female sex ratio (Fig 1).

**Feeding trials**

In experiment 1, all groups had similar growth rates and survival (Table 1). Growth performance expressed as the mean weight was uniform among dietary treatments for the 42 days of feeding (Fig 2). The evaluation of the final sex ratio proved no deviation among different treatments in comparison to the control group (96.6 ± 3.3% females). However, in the MT group the final male ratio was 100% (Fig 3).

In experiment 2, although not significant, the SPIRO diet fed fish had numerically the smallest mean weight after 28 days of feeding than in any other treatments (Fig. 4). The SPIRO diet negatively affected the survival (49.5 ± 15%) of tilapia juveniles compared to the other treatments (94.3 ± 5%) (Table1). The highest mortality level was observed during the first 2 weeks of feeding (Fig 5). No significant differences were observed in the final mean body weight (0.71±0.07 g) and SGR (10.6 ± 0.2 %/day) among all dietary groups (Table 1). Our results indicated that the sex ratio of genetically all-female tilapia is not affected by the inclusion of the tested plant extracts. However, MT and SPIRO supplemented diets fed fish had significantly ( $P < 0.05$ ) increased male ratios, 100% and 75%, respectively (Fig 6).

**Discussion**

The selected plant aqueous extracts apparently do not contain the presumptive compounds that could exert endocrine disruptive activity given that no response to deviation from its original genetic sex ratio was observed in juvenile tilapia. Although evidence from mammals indicates that specific extracts exhibit aromatase inhibitory properties (Moundipa et al., 1999), the same species of plants and a similar preparation of extracts did not exert a sex reversal in fish. In other words, effects similar to synthetic aromatase inhibitors such as ATD (Guigen, 1999) and fadrozole (Kwon et al., 2000) could not be demonstrated. The feeding studies frequently follow *in vitro* tests, and at present, there is evidence of some potential activity of plant extracts as aromatase inhibitors. For instance, extracts of green and black tea did promote the inhibition of aromatase in JAR cells *in vitro* (Monteiro et al., 2006), and the administration of P-60 green tea catechins to rats elevated levels of endogenous testosterone and inhibited aromatase in an *in vitro* essay with human placental cells (Satoh et al., 2002). One of the possible explanations that sex reversal effect was not observed in the case of the tested plant extracts in the present experiments could be related to the mixture of the desired and undesired compounds. Lee et al. (2005) observed that

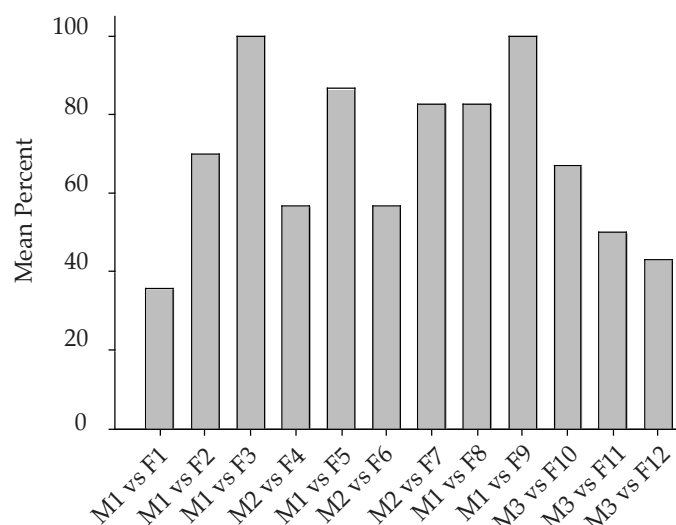


Figure 1: Final mean percentage of females observed in experiments with individual pairs of fish. Progenies of various combinations of males (M1-3) and females (F1-12) were examined for gonad sex after at least 42 days of weaning (n = 30 fish per pair)

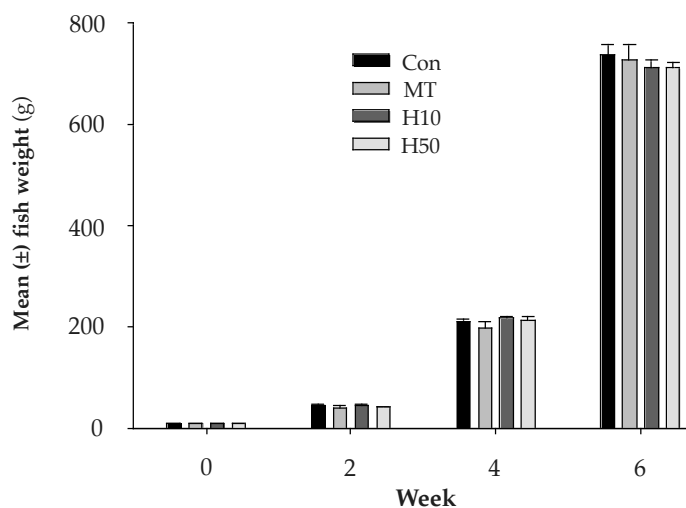


Figure 2: Progress in mean fish weights observed in experiment 1 (all-female tilapia) at 2, 4 and 6 weeks of weaning. Note that the treatment with MT was withdrawn at 4 weeks.

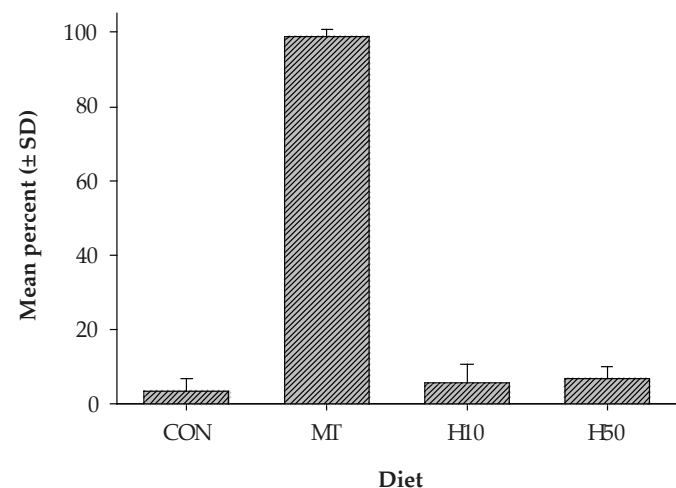


Figure 3: Final male percentage observed in experiment 1 (mean ± SD). Thirty progenies were examined per tank, 90 fish per dietary treatment. Fish were sexed at the age of 48-54 days

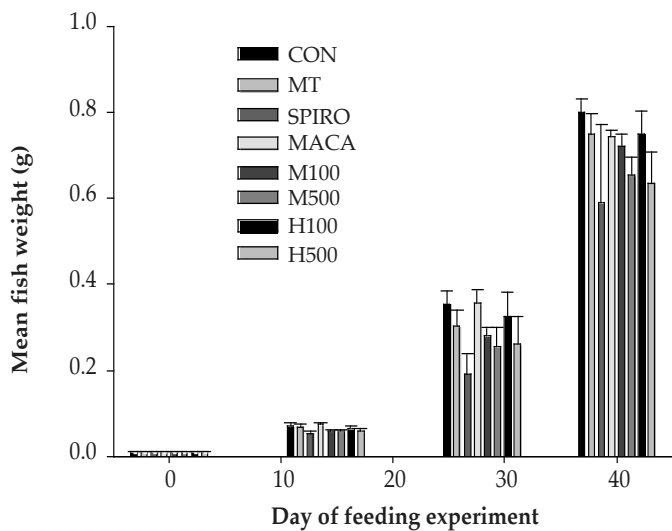


Figure 4: Progress in mean fish weight observed in experiment 2 (all-female tilapia). Values are mean  $\pm$  SD of triplicate groups per dietary treatment. No statistically significant differences were noticed

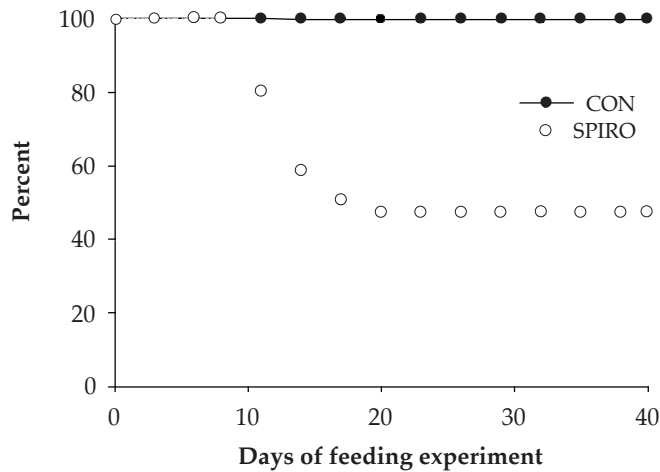


Figure 5: Progressive in cumulative mortality in tilapia juveniles fed diet containing no supplements, control (CON) and fish fed spironolactone (SPIRO, 10 g / kg) in experiment 2.

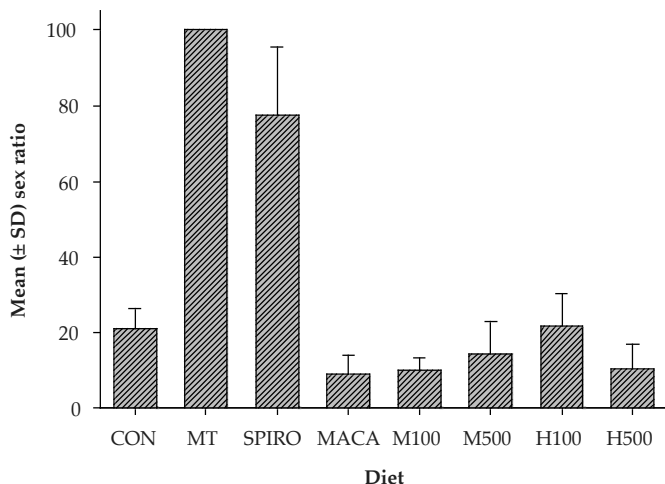


Figure 6: Final sex ratio per dietary treatment (mean  $\pm$  SD). 79 - 80 fish were examined per treatment. Fish age at sexing was 55-60 days.

among several fractions of maca extracts, there are chemicals with growth-promoting and growth-inhibiting activity. Similar phenomena may have been observed with the aqueous extract and fractionation and purification is required in further studies. This information available from fish may require re-evaluating existing results in rats (Gonzales et al., 2001) in respect to the androgenic effects of maca. Contradicting results were also published recently that question maca's androgenic properties, given the lack of an effect on inducing androgen mediated responses in the prostate cancer cells after using a series of solvent fractions (Bogani et al., 2006).

Spironolactone proved to a certain extent its potential to be used to reverse the genotypic sex of the gonad in tilapia. Aromatase inhibition activity for spironolactone was initially reported by Carr (1986), and subsequent reports on the effect of this synthetic steroid as an agent for paradoxical masculinization in mosquito fish (Howell et al., 1994) provided some additional evidence. Our results, where a 75% masculinization rate was achieved, are significant. Still, given its apparent high toxicity at the used concentration in experiment 2, and the observed mortality, it contrasts with our significant finding. Therefore, more testing is required to validate the appropriate dosage, timing and duration of the spironolactone treatment.

#### ANTICIPATED BENEFITS

We have found that masculinizing juvenile Nile tilapia by dietary treatment with spironolactone can be an interesting alternative to using MT. This chemical needs to be further examined bearing in mind its toxicity and conditions of treatment (dose, duration, fish size) need to be optimized. Long-term feeding effects must be analyzed. The phytochemical purification procedure requires refinement but it is a significant finding that *in vitro* effects in cell cultures addressing possible mechanism of sex steroid mimics cannot be directly confirmed in live fish.

#### ACKNOWLEDGMENTS

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

## USE OF PHYTOCHEMICALS AS A NEW METHOD TO SEX-REVERSE NILE TILAPIA AND TROPICAL GARFISH. PART II: INITIATION OF FEEDING AND GONAD DIFFERENTIATION IN LONGNOSE GARFISH

*Twelfth Work Plan/ Fish Nutrition and Feed Technology 3 (12FNF3b)*

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### ABSTRACT

Longnose gars (*Lepisosteus osseus*) were used as a surrogate species for the tropical gar (*Atractosteus tropicus*) in the present study in order to focus on early gonad development and sex differentiation. There is evidence that differentiation of gonads and gamete release pathways are similar among Lepisosteidae. We carried out controlled reproduction of hormonally induced longnose gar and following embryonic development reared the larvae of this species using a combination of live and formulated diets. The description of the ontogenetic development of gonads in gar is presented to establish the relationship between fish size (age) and gender. Histological appearance of the gonads was characterized by early signs of morphological structure of primordial germ cells as early as prior to the first exogenous feeding (23.5 mm total body length) and presumptive seminiferous tubules (presumptive masculine characteristic) in fish of 107 mm total length. We have concluded that garfish larvae/juveniles can be effectively adapted to consume dry formulated diets at early stages, after an initial feeding of 2-3 days using live food (brine shrimp nauplii). We recommend that diets supplemented with 17 $\alpha$ -methyltestosterone (MT) should be used from 14 days after hatching. Furthermore, the hormonal treatment could be included within the first 3-6 week of exogenous feeding.

### INTRODUCTION

In garfishes (Lepisosteidae), females grow twice as large as males (Garcia de Leon et al., 2001; Love, 2002), so it would be desirable for aquaculture to produce all-female stocks. There is an increasing demand by fish producers in southeastern Mexico to develop aquaculture methods for a native species such as tropical garfish, thus attracting local markets and allowing possible exports from Tabasco and Chiapas states. Therefore, alternative species to Nile tilapia, available in the region since early 70's, need to be seriously considered. Native to Mexico, tropical gar is of high local demand and cultural value (Mendoza et al., 2002). Preliminary data gathered in a series of experiments in UJAT suggests that this fish is particularly suitable for high density culture, is very tolerant to water quality fluctuations, and in association with high fecundity, relatively easy to reproduce in captivity (Contreras, W, personal communication).

Contreras et al. (2003) reported an increase in the percentage of females (51–58%) in tropical garfish fed estradiol-enriched *Artemia* nauplii for 20–28 days, in comparison to 10-15% females in control groups. This low percentage of females overall is puzzling. Tropical garfish larvae are characterized by fast growth, 1 mm/day until day 15 after hatching and 2.5 mm/d

between days 15 and 30 (Aguilera et al., 2002). Further studies related to growth and sex differentiation in tropical garfish are desirable and feminization of reared fish with natural estrogenic substances of plant origin may be a viable option for producers. The goal of the proposed project is to further document gonadal sex differentiation (see Lin et al., 1997) in garfish during early ontogeny. There is evidence that differentiation of gonads and gametes release pathways is similar among three species of Lepisosteidae (Ferrara and Irwin, 2001). Therefore, the aim of our study was to describe ontogenetic development of gonads in longnose gar. Fish size (age) when the gender of the gonads is histologically distinguishable is important for the determination of the stage at which hormonal masculinization in this species is possible. In this case the longnose gar was treated as a surrogate species for tropical gar *Atractosteus tropicus*, a fish of aquaculture potential in Mexico.

MATERIALS AND METHODS

Broodstock and artificial spawning

Longnose gar broodstock was obtained by electrofishing with the cooperation of the Ohio Division of Wildlife and the Aquatic Ecology Laboratory at the Ohio State University in Sandusky River, OH, in March 2005. Fish were acclimated to laboratory conditions for several weeks and fed live prey. Presumptive females ( $n=2$ ) and males were identified and separated into groups of one female and three males. To induce the final maturation, fish were first injected with a hormone priming dose, followed by a resolving dose of OVAPRIM® 8 hours later. Eggs were released by one female, and sperm was released by only 2 out of 15 injected males. The ovulating female was stripped of eggs 48 h after a resolving dose and then several times within the following 24 h. A male garfish was sacrificed, and the sperm preparation was obtained from macerated testes to assure insemination.

Feeding

A feeding trial was setup when larvae were 11 days old (initial weight 37.3 mg, length 23.5 mm), and the fish were distributed into nine 35 L glass aquaria with 11 fish per tank. Control groups were fed with live *Artemia* nauplii. In two other treatments we attempted to provide a formulated commercial diet (AgloNorse Ewos, Norway; 59% protein 16% lipids) for two days. However, no feeding was observed, therefore all groups were offered live *Artemia* nauplii for the four following days. The second attempt of weaning from live food into a commercial diet (Aglonorse) (three tanks) was carried out when fish were 37.4 mm total length. At the same time a feed (Aglonorse) with 60 mg/kg 17 $\alpha$ -methyltestosterone (MT) was offered to another three replicate groups of fish. Fish were fed *ad libitum* in 1–2 h intervals, 12 h daily, for 20 d. Growth as final mean weight and total length and survival was recorded at the end of this phase. After completion of the MT treatment (20 d), fish continued to be fed either live feed (juvenile Nile tilapia) or a commercial diet (Silver Cup 42–15%; protein-lipid ratio) *ad libitum* in their initial tanks for four additional weeks. Fish were then combined into a single group for each dietary treatment, and stocked into three 400 l-tanks. Live feed group was offered live tilapia juveniles as food, and two other treatments (MT or not MT) received Silver Cup commercial diet provided with a belt feeder for 12 h a day.

Gonad development

Several fishes were fixed for histological analyses at the time of initiation of feeding with exogenous food, and at the time of completion of the MT treatment. Fish were processed by standard histological methods (paraffin embedding, H+E staining). The following specimens were chosen for the present study from live food fed group, with one exception: 23.5 mm TL ( $n=3$ ) (11 days post hatching; dph; prior to exogenous feeding); 44 mm total length (TL) (20 dph); 68 mm (39 dph; AgloNorse diet), 107 and 140 mm (98 dph; 3 months old) ( $n=1$ , each size).

RESULTS

Induced spawning

Approximately 400 eggs were obtained from the injected

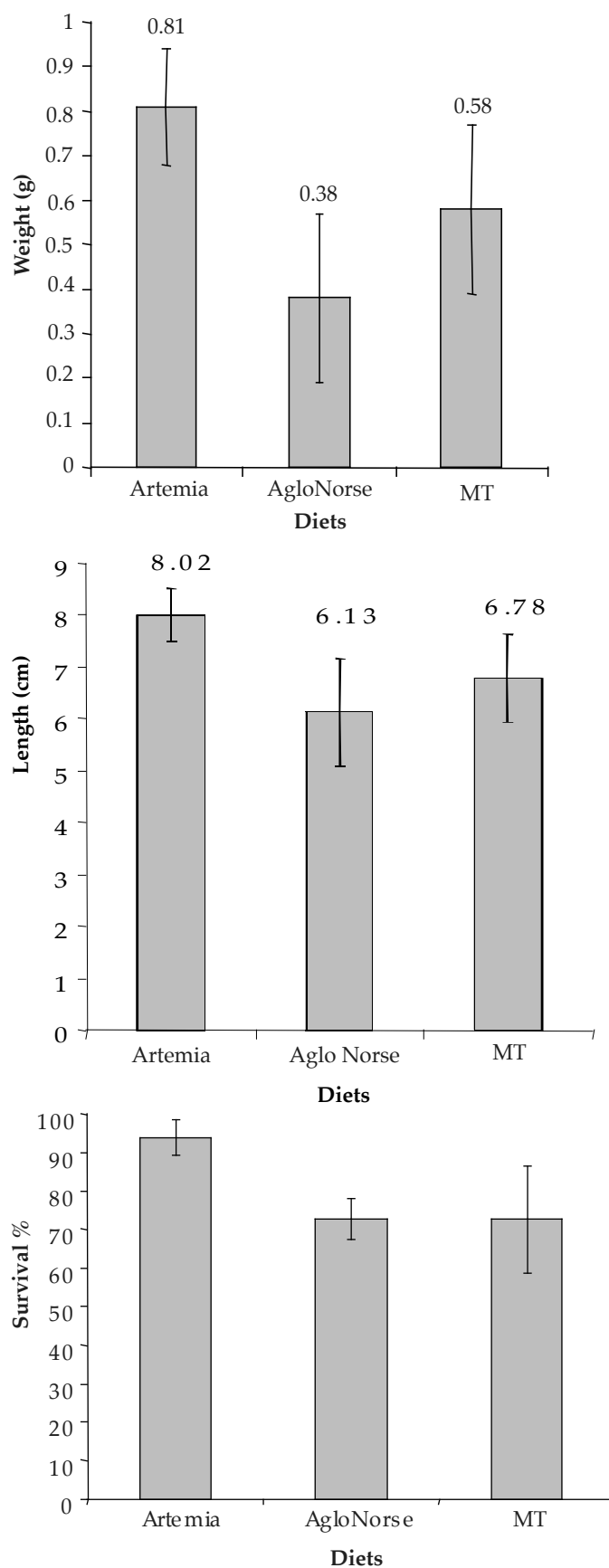


Figure 1: Mean body weight (A), total body length (B), and survival (C) at the +time of completion of MT treatment (20 days for feeding). There were no significant differences observed among treatment groups although numerically fish fed live food were twice as large as those fed a formulated commercial feed.

female, viable eggs were mostly obtained during the first stripping, and fertilization was negligible in the following partial ovulations. After incubation for six days at 18°C, 106 larvae hatched.

**Feeding**

Garfish showed no significant differences in body weight and length among dietary treatments due to a large variation in size (Fig. 1a, b). Some emaciated fish were removed from the AgloNorse diet fed groups and counted as mortalities. However, overall survival was high (Fig. 1c). Attempts of cannibalism were also observed on several occasions, particularly in the live *Artemia* fed group where mortality was solely due to cannibalism.

**Gonad development**

The body of the specimens prepared for histological analyses were divided into three sections as described earlier (Fig. 2a) and will be referred to as A, B, and C. This was accomplished to precisely locate the gonads at the very early stages of development as well as to document possible difference in structures or functions along the body axis. Gonadal crests (GCs), as undifferentiated gonads (Fig. 2b), were found in garfish as early as 11 dph at 23.5 mm (TL). Histological studies have shown that GCs are situated on both sides of the dorsal mesentery, and located under the opisthonephros in the middle part (B) of the coelomic cavity. They consist of primordial germ cells (PGCs) surrounded by somatic cells. PGCs were easily identified by their large size, ovoid shape and large nuclei with

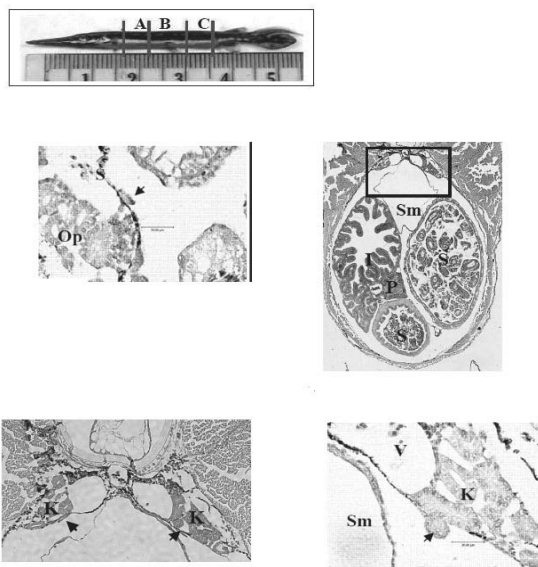


Figure 2: Body of garfish divided into 3 segments (A, B, C) (a). Section in the middle of the body cavity. Gonadal crest (arrowhead) in fish at 23.5 mm TL (11 dph) without germ cells and epithelia; Op – opisthonephros, S – splachnoleura (b). Position of gonadal crests in body cavity. Fish at 44 mm TL (20 dph); Sm – swim bladder, I – intestine, S – stomach, O – pancreas, K – kidney, arrowheads – gonads (c, d). Gonadal crests with primordial germ cells from fish of 44 mm (PGCs, arrowheads) in the middle part of body cavity (B), visible on the both sides of mesentery; V – vena caudalis, K – kidney, Sm – swim bladder (e).

a distinct outline (see also criteria described in Lin et al., 1997). During growth and development of the fish, gonadal crests progressed into the stage of gonadal folds that are attached to the dorsal body wall by a thin mesogonadium (Fig. 2c). Gonadal folds were observed for the first time in garfish of 44 mm TL (20 dph). The characteristic features of the folds were the presence of the regionally differentiated epithelium (Figs. 2d, e). On the lateral side of the cross-section, a layer of the high columnar cells covered the gonadal fold. On the medial side, a flat epithelium was present (Fig. 2e).

At the completion of the feeding experiment (39 dph) (Fig. 1) the appearance of the urogenital system was macroscopically undefined (Fig. 3a). Furthermore, histological sections of the posterior part of the urogenital organ revealed that these structures could not be associated with either sex at this stage of ontogenesis (fish size).

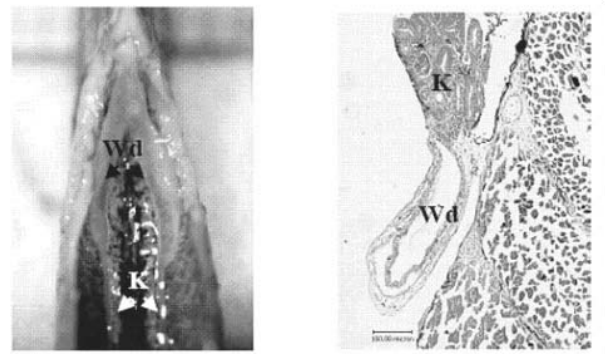


Figure 3: Wolffian ducts (Wd and black arrowheads) of longnose gar, segment C, fish at 68 mm TL (39 dph), K and white arrowheads – kidney; a) macroscopic image, b) microscopic picture.

Fish of sizes 107 and 114 mm, displayed gonads that contained some tubules with aligning cuboidal epithelium (Fig. 4). These canals, present inside gonadal folds, seem to be presumptive seminiferous tubules. When completely formed these features will become the means of gamete release pathways in males. In adult males the sperm ducts will be connected with the excretory part of the kidney to form the urogenital system (Ferrara and Irvin, 2001).

Mean weights after 71 days of continuous rearing of longnose gar in the present study were as follows: live feed group 15.2 ± 3.6 g, and 12.4 ± 3.0 g and 13.9 ± 3.8 g for the no MT and MT group, respectively.

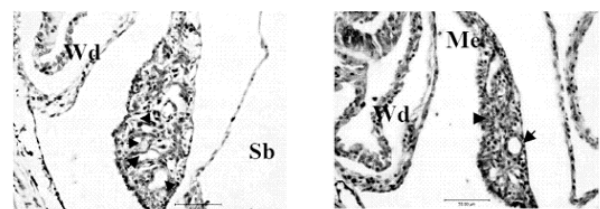


Figure 4: Distal part of body cavity. Cross-section of the gonadal folds in fish at 107 mm TL (3 months old) with visible presumptive seminiferous tubules (arrowheads); Wd – Wolffian duct; Sm – swim bladder (a). Middle section of the body cavity. Cross-section of the gonadal folds in fish at 139.9 mm TL (3 months) with differentiated mesogonadium (Me) and epithelia outside and inside gonad (arrowheads); Wd – Wolffian duct (b).

## DISCUSSION

We conclude that garfish larvae/juveniles can be effectively transitioned from live brine shrimp nauplii to accept formulated diets at early larval stages. This is a promising finding, parallel to observations made by Contreras (personal communication) with tropical garfish and suggests that formulated diets can be used as a method to deliver substances affecting gonad differentiation (masculinization or feminization) in these species. The hormonal treatment could be included within the first week of exogenous feeding.

Gonads are highly specialized organs, as they constitute the environment for the multiplication and differentiation of germ cells into spermatozoa and/or egg cells (Timmermans, 1987). The presence of the presumptive germ cell structure, similar to gonadal crests and gonadal folds in garfish at size of 23.5 and 44 mm, respectively, were also observed by Allen (1911). The evidence provided by Allen (1911) was, however, incomplete as gonads were described in fish of 24 and 110 mm TL, and the intermediate sizes were not examined. We provide here the first evidence of the formation of the gonad based on the differentiation of PGCs within the envelope of somatic cells.

The significant finding of the present study is the notion that the somatic part of the gonad may undergo sexual differentiation earlier than the germ cells. It is known that PGCs are kept in a resting stage whereas all other tissues multiply actively (Timmermans, 1987). Therefore, the presence of a seminiferous seminal duct in fish at 107 mm is the signature of maleness and suggests that gonadal sex differentiation in garfish occurs early in age and size. We illustrate in Fig. 4 that the gonadal gender, in the present case toward testis, possibly exists in longnose garfish at the age of three months. This result also illustrates a difference between garfish and white sturgeon, which are closely related as members of the class Actinopterygii. Gonadal sex differentiation in fish from the order Acipenseriformes occurs between 1 and 5 years of age (Eenennaam and Doroshov, 1998). For the most part, the teleosts possess rudimentary gonads which undergo a period of slow somatic growth with apparent histological differentiation and an accelerated increase of PGCs (Lin et al., 1997). Germinal activity, which can last for months (eel, *Anguilla*) or years (sturgeon, *Acipenser*), depends on the fish species (Grandi and Colombo, 1997; Strüssmann and Nakamura, 2002).

Because of the presence of gonadal crests with PGCs in garfish at a size of 23.5 mm, and the early differentiation of gonads, we conclude that hormonal treatment (masculinization) can be applied in garfish from the time of beginning of exogenous feeding. A particular aspect of this result is the finding of male differentiation occurring earlier than in the female, although this requires more individuals to be examined. Description of intersex gonads in sturgeon (Jackson et al., 2006) also poses the question of morphological differentiation of the presumed hermaphrodites. In general, the assumption that the differentiation into the male gender occurs at an early stage contradicts earlier assumptions that "undifferentiated gonads" proceed from the beginning through an all female phase or intersex phase prior to actual differentiation into ovaries or testes (Strüssmann and Nakamura, 2002). In this case, which is commonly referred to as "differentiated" gonochorism, ovarian and testicular differentiation proceeds directly from the undifferentiated gonads even though both events may not occur simultaneously (Strüssmann and Nakamura, 2002; Jackson et al., 2006).

## ANTICIPATED BENEFITS

Present findings are significant because they provide the evidence for the first time that rearing of longnose gar can be achieved in controlled conditions and it is an excellent surrogate species for tropical gar. These findings on the dynamic of gonad differentiation are applicable to tropical gar. In further studies we will focus on the description of the morphological development of testis

in masculinized specimens, however, this will require sampling individuals of 2-3 years of age to confirm functional maleness (sperm production).

## ACKNOWLEDGMENTS

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# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## WATER QUALITY MONITORING AND IDENTIFICATION OF POLLUTION SOURCES LEADING TOWARDS CLASSIFICATION OF BIVALVE GROWING WATERS

*Twelfth Work Plan, Aquaculture and Human Health Impacts 1 (12AHH1)*  
Abstract

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### ABSTRACT

Two major bay systems in Mexico, which are the focus of collaborative efforts for international integrated coastal zone management efforts, Bahia Santa Maria (BSM) in Sinaloa and Marismas Nacionales in Nayarit are home to growing oyster industries. Oyster farming in Nayarit has a 30-year history while women's producer groups in BSM are just beginning. In Nayarit, farming has been demonstrated to be a viable alternative to fishing for coastal communities, particularly because women have a high level of participation, both in production and processing. Two oyster species are commonly cultivated along the Pacific coastline. *Crassostrea gigas* seed is imported from the US and used for remote setting in Mexico, mainly along the northern extent of the coast. A native species, *Crassostrea corteziensis*, has sufficiently high levels of localized spat set to support a limited industry, mostly confined to Nayarit and two newer farms in Sinaloa. There is also growing interest in other native bivalve species, so it is hoped that the outcomes of this effort to improve shellfish sanitation will have wider benefits.

Among the obstacles to progress is the questionable water quality in bivalve growing areas. Increasing populations and pollution in coastal areas threaten the safety and economic viability of the growing oyster culture industry. Opportunities also exist as many areas are still relatively pristine and produce a high quality and safe product. Previous work found that the ability to assure product safety, transport, and market in other areas and to produce value-added products could greatly increase the direct socio-economic benefits this industry provides to coastal communities. There is also a possibility that shellfish could be exported to the US, as two Mexican farms are already doing, if water quality and the regulatory framework is such that growing areas could be classified according to US standards.

Because both the existing Mexican and US protocols and standards would require at least one year of intensive water quality monitoring to classify a growing area and because these areas are extensive, attempting to classify them is not a trivial task and resources do not exist to undertake large-scale monitoring efforts. The first, more feasible option is to conduct rapid assessments that include shoreline surveys and preliminary water quality monitoring to eliminate any areas which could be conclusively barred from consideration and to identify the areas most likely to be able to meet standards in the future. Once these areas are identified, intensive monitoring efforts could then be conducted in a more cost-effective manner in narrowly targeted geographic areas of the two bays. Participants in this work include Universidad Autónoma de Sinaloa, Pacific Aquaculture and Coastal Resources Center/University of Hawaii at Hilo, University of Hawaii Sea Grant College Program, Ecocostas, Coastal Resources Center/University of Rhode Island, Louisiana State University Sea Grant College Program, CESASIN, CREDES, Autonomous University of Nayarit, oyster farming cooperatives of Nayarit, and women's groups of BSM. Investigation 12AHH2, "Outreach and Planning for Implementation of Bivalve Growing Areas Classification and Related Sanitation Action Items," is complementary to this investigation and constitutes a planning, regulatory, and outreach component.

Working closely with local stakeholders, progress to date includes identification of the oyster growing sites and water quality monitoring sampling stations. Two workshops have also been held with the full range of stakeholders concerned with this work including aquaculturists, federal and state government representatives, researchers, students, and oyster vendors to present the objectives of the work, preliminary results, and to build constituency for development of an integrated management plan for the estuary. First steps have been taken towards drafting of a management plan with full involvement of the stakeholders.



# AQUACULTURE CRSP 24<sup>TH</sup> ANNUAL TECHNICAL REPORT

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## OUTREACH AND PLANNING FOR IMPLEMENTATION OF BIVALVE GROWING AREAS CLASSIFICATION AND RELATED SANITATION ACTION ITEMS

*Twelfth Work Plan, Aquaculture and Human Health Impacts 2 (12AHH2)  
Abstract*

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### ABSTRACT

Culture of oysters and other bivalve species is a growing opportunity for aquaculture along the Pacific Mexican coast. Bivalve culture, and the need for sanitation protocols to assure the safety and quality of the shellfish products are relatively new topics for the Pacific Mexico region. As efforts to diversify aquaculture through strengthening of shellfish culture are underway and as consumer awareness of the potential dangers of consuming aquatic products increases, measures to assure the production of safe shellfish and other aquaculture products are needed. This activity is linked to Investigation 12AHH1 "Water Quality Monitoring and Identification of Pollution Sources Leading towards Classification of Bivalve Growing Waters," and will be aimed at disseminating the findings of that study and raising awareness of the issues associated with shellfish sanitation and other aquaculture products. Researchers, extension agents, and government officials will then work together to identify strategies and resources to implement recommendations stemming in part from Investigation 12AHH1 as well as the outcomes of the Year 10 work. A wide range of environmental, community, and product sanitation issues were identified during study of finfish, shellfish, and shrimp operations.

Particular attention will be paid to monitoring and classification of shellfish growing waters and actions targeted towards mitigating major sources of pollution that are affecting aquaculture as a whole. Previous work in Bahia Santa Maria (BSM) by the members of the Sinaloa working group has already developed tools and strategies that have led to positive improvements in community sanitation and water quality. Expansion of these efforts within the BSM system and replication in Nayarit would contribute to an increased probability that shellfish growing areas could be classified as approved and that other aquaculture sanitation problems could be addressed.

Specifically, this work will raise awareness among key institutional and community stakeholders about the major issues associated with aquaculture sanitation. Stakeholders will be educated about the technical and legal requirements for safe production of bivalves. Findings, outcomes, lessons learned, and strategies will be disseminated to the authorities and key stakeholders so that joint development of strategies and resources to implement programs for classification of shellfish growing waters and other strategies related to community sanitation and water quality can take place. An implementation plan for the above mentioned topics will also be developed. Participants in this work include Universidad Autónoma de Sinaloa, Pacific Aquaculture and Coastal Resources Center/University of Hawaii at Hilo, University of Hawaii Sea Grant College Program, Ecocostas, Coastal Resources Center/University of Rhode Island, Louisiana State University Sea Grant College Program, CESASIN, CREDES, Autonomous University of Nayarit, oyster farming cooperatives of Nayarit, and women's groups of BSM.

Two workshops have also been held with the full range of stakeholders concerned with this work including aquaculturists, federal and state government representatives, researchers, students, and oyster vendors to present the objectives of the work, preliminary results, and to build a constituency for development of an integrated management plan for the estuary. First steps have been taken towards drafting a management plan with full involvement of the stakeholders. Awareness has also been raised among the oyster farmers and government representatives as to the need for a shellfish sanitation plans and the requirements of such a plan to assure product quality within the Mexican regulatory framework and secondarily, within that of the US, anticipating future export possibilities.