Aquaculture
Collaborative Research Support Program

ELEVENTH WORK PLAN, PART II

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

Contents of the Eleventh Work Plan, Part II

Fish Nutrition and Feed Technology

Aquaculture and Human Health Impacts

Best Practices for Management of Aquaculture Pond Soils in Thailand

Environment Impacts Analysis 1 (11.5EIAR1)/Study/Thailand

Investigators

Claude E. Boyd	US Principal Investigator	Auburn University, Alabama
Mali Boonyaratpalin	HC Principal Investigator	Thailand Department of Fisheries, Bangkok, Thailand
Idsariya Wudtisin	Graduate Student	

Objectives

1) Evaluate pond soil data collected in Thailand and determine major similarities and differences related to culture species.
2) Develop best management practices for pond bottom soil management in Thailand, and evaluate the degree of adoption and effectiveness of the practices.

Significance

Sustainability and protection of the environment are major issues in aquaculture (Black, 2001). Unsustainable projects are economically damaging, but often, they are the aquaculture endeavors that do the most 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 should be given 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).

Recent studies funded by Aquaculture CRSP and conducted in Thailand by Auburn University researchers and collaborators in the Thailand Department of Fisheries have provided much data on the physical and chemical properties of bottom soils in ponds for culture of black tiger shrimp (*Penaeus monodon*) in coastal areas, low salinity culture of black tiger shrimp in inland areas, and production of tilapia (*Tilapia* spp.), freshwater prawn (*Macrobrachium rosenbergii*), catfish (*Clarias* spp.), and carp (*Puntius* spp.) in freshwater areas.

Because of high production levels in Thai aquaculture, deterioration of pond soil condition is a common problem. The information on soil characteristics and pond management procedures accumulated in the aforementioned studies can be used to develop best practices for pond soil management in Thailand. These best management practices (BMPs) will be valuable in improving the prospects for sustainable and environmentally-responsible pond aquaculture in Thailand and other nations.

Quantified Anticipated Benefits

The best management practices will provide a means of transferring the best available information on pond soil management in a simple form that can be understood easily by producers. The specific expected benefits are as follows:

1) Development of guidelines for identification of soils with properties suitable for aquaculture ponds.
2) Preparation of BMPs for mitigating limitations of site soil properties and for maintaining good bottom soil quality.
3) Preparation BMPs to prevent negative external impacts related to pond soil management.

Research Design and Activity Plan

Pond Facilities: No ponds are needed for this research. However, pond soil characteristics have been determined for many ponds in most major aquaculture areas of Thailand (Boyd and Munsiri 1997; Boyd et al., 1997, 1998, 1999, 2000; Wudtisin et al., 2004; Thunjai et al., 2004). This database will provide information needed in developing the BMPs.

Research Plan and Methodology: The US PI has considerable experience in developing BMPs for pond aquaculture (Boyd, 1999, 2003; Boyd et al., 2001, 2003). The procedure used to develop BMPs involves determining the negative impacts that result from a particular activity and developing practices (BMPs) for preventing or mitigating the negative impacts. For example, suppose there is a layer of potential acid-sulfate soil at a depth of 1 m beneath an area where ponds are to be constructed. The practice for preventing excessive acidity in ponds is to construct ponds in a manner that this soil layer is not disturbed and is not exposed in the bottoms of ponds or used in construction of pond embankments. Another good example is ponds located in an area with bottom soils of a pH of 5. Such soils must be limed regularly to maintain pH above 7. Of course, the BMP will provide information on pH measurement and liming practices.

Pond soil BMPs also will reduce negative effects of pond aquaculture on adjacent land and nearby water bodies. The total suspended solids concentration is the water quality variable in pond effluent most likely to exceed an acceptable level (Boyd and Tucker, 1998). High concentrations of suspended solids cause elevated turbidity and increased sedimentation rates in receiving water bodies. Thus, pond soil BMPs will include ways of reducing the concentration of total suspended solids in pond effluents. For instance, the BMPs will recommend that ponds are drained in a manner to minimize sediment resuspension, outlet gates or drains are closed in empty ponds to avoid discharge of turbid water after heavy rains, and outlet canals are designed to resist erosion during effluent discharge. The practice of washing pond bottoms with jets of water from high-pressure hoses, as sometimes done in Thailand, will be discouraged to prevent pollution of receiving waters. Moreover, disposal of pond sediment on nearby land can cause deterioration of vegetation at the disposal site, and runoff from sediment piles may increase turbidity and sedimentation in nearby streams or other aquatic habitats. This study will include the design of BMPs to lessen negative, external impacts.

The database mentioned above on pond soils in Thailand will be evaluated to determine the main soil limitations (Hajek and Boyd, 1994) that affect Thai aquaculture. Information on pond soil management (Boyd 1995) will be used to formulate management practices for mitigating the limitations. The database also contains information on changes in pond bottom properties over time and the usual practices applied in pond management during production of major culture species. This information will be used in identifying negative changes that may occur in pond soils as a result of aquaculture and BMPs to prevent these changes will be formulated. These BMPs will assure that good bottom soil quality can be maintained over many years of pond use.

The graduate student for this study is conducting analyses of pond soils from catfish, carp, and freshwater prawn ponds. She will use this information in her dissertation as well as develop the BMPs with assistance from the US PI. The BMPs will be finalized in early 2005 following discussions with the host country PI, other biologists, and fish farmers. They will be translated to Thai by the student and distributed to fish producers by the Thailand Department of Fisheries.

Implementation of soil BMPs will require farmers to make changes in culture system infrastructure, operations, and management techniques. The effectiveness of these changes will be reflected in prevention or reduction in negative impacts on infrastructure and nearby land and water. The US and host country PIs will develop a survey document consisting of a checklist of items to be inspected and questions to be asked of farmers. In early 2006, a sample of 10 to 15 farms in central Thailand will be selected by local biologists of the Department of Fisheries. The PIs will travel to these farms and conduct the evaluation of BMP adoption and effectiveness with aid of the survey document.

Statistical Analysis:
This study does not focus on measurable quantities that can be subjected to statistical analysis.

Regional Integration

The project will integrate well into the regional plan. Pond aquaculture in Thailand is similar to that of other Southeast and South Asian nations. Thus, the BMPs can be applied in other Asian nations. Moreover, many of the BMPs should be useful in pond aquaculture in other regions.

Schedule

The tentative schedule follows:

November 2004: Begin project. Evaluate pond soil data and production methods to identify site soil limitations and possible negative impacts.

December 2004: Student will finish portion of dissertation dealing with carp, catfish, and freshwater prawn pond soils.

January-March 2005: BMPs will be formulated, translated to Thai, and distributed to fish farmers.

April-November 2005; Manuscripts on development of the BMPs will be prepared for submission to the *Thai Fisheries Gazette* and to an international journal. The host country PI will respond to questions from farmers about implementation of BMPs. The US PI will be involved through e-mail correspondence.

December 2005: Student will complete dissertation and graduate.

March 2006: US PI will go to Thailand to assist with evaluation of degree of adoption and effectiveness of BMPs.

30 April 2006: Termination of project (final report).

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Establishment of Links with Chinese Institutions in Collaboration on Aquaculture and Environmental Impacts

Environmental Impacts Analysis 2 (11.5EIAR2)/Activity/China

Investigators

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

Objectives

1) Establish links wiath Chinese institutions for future CRSP research.
2) Identify potential CRSP sites in China and to conduct preliminary site evaluation.
3) Identify environmental problems caused by aquaculture/fisheries activities and develop researchable topics.

Significance

Aquaculture CRSP has conducted research/outreach activities in Asia for more than two decades. However, it has not been expanded to China, which is the largest aquaculture producer in the world.

Aquaculture and culture-based fisheries have been developed and intensified rapidly in China since late 1970s, and have played important roles in the livelihood of Chinese farmers through employment, income generation, and food security. China produces about 70% of the world's farmed aquatic products. However, environmental impacts related to inland aquaculture and culture-based fisheries have caused serious concerns. Lack of environmental awareness and environmentally friendly technologies have polluted most public waters such as rivers, lakes, and reservoirs. Many dams are constructed on small streams or large rivers to create artificial reservoirs for various purposes including fish production. The impacts of dams and related fishery activities on natural aquatic ecosystems and native biodiversity urgently need to be addressed. With intensification of aquaculture systems, heavy uses and abuses of chemicals and drugs are concerns for food safety and sustainable use of those waters for fisheries production. China also has the largest number of exotic aquatic species in the world. Potential impacts of fishery development on biodiversity should be addressed, and appropriate control mechanisms need to be established. However, such environmental concerns have not been fully addressed through research and education at various levels. Therefore, the purpose of this study is to identify and address environmental issues related to aquaculture and fisheries activities for promoting sustainable development of inland aquaculture and cultured-based fisheries in China.

Anticipated Benefits

This study will be important to increase public awareness on aquaculture-related environmental issues through education, research, and outreach in China.

Research Design

1) Establish linkages between AIT and Chinese institutions including academic and governmental institutions;
2) Hold a two-day expert consultation meeting including 20-25 experts from various institutions as well as members of the CRSP PMO to review environmental issues in aquaculture and fisheries in China, and identify and prioritize researchable topics;
3) Support 1-3 graduate students each from an identified partner institution to conduct thesis research on different environment issues jointly supervised by AIT and Chinese researchers; and
4) Support 1-3 Chinese scientists each from the identified partner institution to attend the World Aquaculture Society Annual Meeting and the Aquaculture CRSP Regional Network Meeting to be held in May 2005 at Bali, Indonesia.

Schedule
September 2004–May 2006.

Report Submission
Expert consultation report will be submitted 30 days after the consultation meeting;
Final report will be submitted 31 May 2006.

Impact of Tilapia, *Oreochromis niloticus* Introduction on the Indigenous Species of Bangladesh, Nepal, and Cambodia

Environmental Impacts Analysis 3 (11.5EIA3)/Experiment/Bangladesh and Thailand

Investigators

Amrit N. Bart	HC Principal Investigator	Asian Institute of Technology
Md. Abdul Wahab	HC Principal Investigator	Bangladesh Agricultural University
James E. Rakocy	US Principal Investigator	University of Virgin Islands
James S. Diana	US Principal Investigator	University of Michigan

Objectives

1) Monitor the change in population structure of small indigenous species of Bangladesh and Nepal in the presence of tilapia in static pond environment.
2) Monitor the reproduction of these indigenous and compare with tilapia
3) Assess competition for food resources between tilapia and local indigenous species
4) Develop recommendations and strategies for the introduction of tilapia in areas where tilapia is not indigenous.

Significance

Tilapia has received significant research attention in the past 20 years, including those provided by CRSP. Culture of tilapia is being promoted as a poor farmer's fish as well as fish with export potential in many parts of Asia. Tilapia is also perceived as an aggressive feeder and prolific breeder even under relatively stressful environments. Despite rapid proliferation of tilapia culture worldwide, several countries continue to remain cautious, as they fear that tilapia may compete with the local indigenous species, causing loss of biodiversity and an ecological imbalance. This fear has been exacerbated by statements such as 'Populations of many indigenous small fish species have declined due to the invasion of *Tilapia niloticus* in our (Bangladesh) water bodies' (IUCN Biodiversity Conference, 2002). Unfortunately many of these claims have been based on anecdotal evidence from the pond experience and survey data (Ameen, 1999). The ability of tilapia, even in the static pond environment, to compete with indigenous locally well adapted species has never been established using scientific approaches. Difficulties in carrying out studies in natural environments where tilapia does not already exist have presented logistical dilemma.

Small Indigenous Species (SIS) of fish are important to rural poor in Bangladesh, Nepal and Cambodia as these species are relatively cheap, consumed whole and contain nutritive values higher than most cultured species (Hossain, 1998). While there are reportedly 260 species of freshwater indigenous species in Bangladesh and 185 species in Nepal (Singh, 1984), only 143 are considered small indigenous species, ranging from 7 to 25 cm as adults (Ali, 1997). These indigenous species have many additional advantages including self-recruitment, fast growth, low trophic feeding levels, high levels of micro-nutrients, including calcium and vitamin A (Thilsted and Hassan, 1993), easy drying and preservation, and cooking by mixing with other food (larger fish require special preparation and a different way of cooking). Rural people of Bangladesh consume between 56 to 73 species of SIS (Minkin, 1993), among which Mola, *Amblypharayngodon mola*, Dhela, *Ostebram*a *cotio cotio* and Puti, *Puntius sophore* are most commonly preferred. These species, similar to tilapia, as they too rely on natural phytoplankton and zooplankton as their primary food sources and are found to breed in static natural water bodies and abandoned ponds (Shafi and Quddus, 1982). Many fear that escaped tilapia if introduced may compete with these species causing not only the loss of biodiversity but also affecting the health of the rural poor who derive year-round high quality micronutrients from these indigenous species.

This study proposes to study the effect of mixed sex and mono sex tilapia introduction in simulated natural ponds along with three important indigenous species and assess the change in population structure and recruitment rates over time.

Anticipated Benefits

Successful completion of this study will:

1) Substantiate the relative competitive ability of tilapia compared to other indigenous locally adapted species in a static pond environment.
2) Determine relative competitive efficiency of monosex and mixed sex tilapia with local indigenous species.
3) Determine the relative growth rates of different species including tilapia and small indigenous species cultured under identical conditions.
4) Help us to formulate strategies and policies for introducing tilapia into in a new environment where tilapia is not commonly produced.

Research Design

Location: Bangladesh Agricultural University, Mymansingh and Nepal Agriculture Research Center (Begnas Lake Station, Pokhera) and RUA (Royal University of Agriculture).

Methods:

Pond Facility: 150m^2 ponds (n=9) will be used to carry out this study in each location.

Culture Period: 20 months. Complete harvests will be done at the end of 10 and 20 months to assess abundence, reproduction and survival.

Stocking Density: 0.53fish/m^2. 20 fish per species (1:1 male to female except in the case of monosex tilapia treatment where males will be hand-sexed)

Test Species: Tilapia, *Oreochromis niloticus*; Mola, *Amblypharayngodon mola* Dhela, *Osteobrama cotio;* and Puti, *Puntius sophore.*

Nutrient Input: None (all ponds will be prepared by liming and fertilization prior to stocking and no additional fertilization or feeding will be applied throughout the study period).

Water Management: Water quality will be monitored according to standard methods (APHA, 1980; PD/A CRSP, 1992).

Sampling Procedures and Schedule: This experiment will run for 20 months in which sampling will be carried out every month. This will allow four to five generations of reproduction and adaptation for all four species. Abundance (total number of each species), individual and batch weight and recruitment will be recorded for each species. Number of larvae, juveniles and adult fish for each species will be monitored through monthly sampling and at harvest. In the case where it is difficult to determine the species because of size, the larvae number and weight of small fish will be estimated, and the fish will be reared in a separate hapa for 30 days or until the species can be determined by visual inspection. After 12 months of stocking, stomach content analysis will be performed on each species to determine diet overlap using Schoener's Index (as described in detail by Wahab, 1994).

Experimental Design, Procedure, Null Hypothesis and Statistical Analysis: This is a completely randomized design in which nine experimental units (150m^2 ponds) will be stocked with equal numbers of adult Mola, Dhela, and Puti in such a way that each pond will not exceed 0.53 fish per m^2.

Tilapia will be introduced after 30 days of stocking indigenous species. While three of these nine ponds will be stocked with mixed sex tilapia (1male:1 female) per pond (n=20) and three will be stocked with hand sexed all male tilapia (n=20), the remaining three ponds will not receive tilapia and will serve as control. Each 150-m^2 pond will hold total of 80 fish (0.53 fish/m^2). These indigenous fish are currently available in Mymensingh (BAU, BFRI, and Tarahara stations).

Null Hypothesis: There is no differences in the population structure of indigenous species over time when either mono sex or mixed sex tilapia are introduced.

Data will be summarized in tables and figures. Deviation of population structure from the control will be compared with a Chi-square test. Mean differences in abundance among species in the monosex and mixed sex treatments will be determined using a simple t-test. Comparison of abundance (adult and/or young) as well as growth rates between four species at each sampling period will be regressed.

Regional Integration

The protection of native species is an important issue facing several countries in South and Southeast Asia where the introduction of tilapia is viewed with skepticism. This work will facilitate in sharing information between these countries. Recommendations resulting from this study will impact many countries where tilapia are already introduced or being introduced. All parties involved (AIT, BAU, and NARC) have strong outreach programs to disseminate findings of research results.

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Building the Capacity of Moi University to Conduct Watershed Assessment

Environmental Impacts Analysis 4 (11.5EIAR4)/Activity/Kenya

Investigators

Mucai Muchiri	HC Principal Investigator	Department of Fisheries, Moi University
Odipo Osano	HC Principal Investigator	School of Environmental Studies, Moi University
Geoffrey Karanja	HC Principal Investigator	Department of Wildlife Management, Moi University
William Shivoga	HC Principal Investigator	Department of Environmental Studies, Egerton University
James Kiyiapi	HC Principal Investigator	Department of Forestry, Moi University
Ruth Kinagwi	HC Principal Investigator	School of Environmental Sciences, Moi University
H. Farrah	HC Principal Investigator	School of Environmental Studies, Moi University
E. W. Tollner	US Principal Investigator	University of Georgia
G. Habron	US Principal Investigator	Michigan State University

Objectives

The overarching objective is to develop a functioning watershed assessment center at Moi University with a GIS-equipped laboratory to integrate water, soils and social indicators needed for assessing the Nzoia river basin and subsequently other important watersheds in Kenya and the surrounding region. A functioning watershed assessment center consists of a GIS-integrated facility that supports the following knowledge areas:

1) Hydrological and water quality determination.
2) Land use identification.
3) Social and Legal assessment.

Significance

A major barrier to socio-economic development in sub-Saharan Africa, including Kenya, is food inadequacy. Some of the more important factors that have led to this status are rapid population growth outstripping food production capacity, post-harvest losses, land degradation that leads to further decline in soil fertility, and climatic changes, particularly periodic droughts and subsequent flooding. This food shortages coupled with high poverty rates that diminish people's ability to afford the ever-increasing food prices, has led to related health problems especially in rural areas. In the effort to meet the required food supplies to feed the growing population, forestlands have been cleared for small scale agriculture. Inevitably, a major challenge to economic developing in Kenya is, therefore, the sustained increase of food production without compromising the integrity of the environment within which that much required food is produced. As such the project seeks to complement other projects that seek to "improve the productivity and sustainability of land use systems in Nzoia, Yala and Nyando river basins through adoption of an integrated ecosystem management approach" through development of on-farm and off-farm conservation practices and increased local capacity (Global Environmental Facility, 2004:3). Desired outcomes include increased biodiversity and reduced erosion (GEF, 2004).

Such a balance reflects decision making regarding risk. People must balance the need for meeting food, housing and health needs with an interest in protecting the environment (Smith et al., 2000). Risk approaches require an integration between positivist and constructivist approaches (Rosa, 1998). Risk not only appears as a function of probability-consequence dynamics, but also as a function of risk perception and responses to risk perception (Cohen, 2000; Rosa, 1998).

We envision Moi University as a regional center for the Nzoia basin management. This center will provide a basis for cooperation and stimulation with and by other projects in Kenya that are on-going in the Njoro basin, where Edgerton University is playing a lead role.

Anticipated Benefits

We anticipate this project to lay the foundation for protecting the Nzoia basin from nonsustainable development. This will be protective of the Lake Victoria and will synergize with in-country efforts to nurture fish production in the region. By building the capacity of Moi University to replicate the effort in other watersheds of Kenya, Moi University can develop a regional reputation as a watershed assessment center that can positively impact the environment in and around Kenya. Physical science skill development would concentrate on developing hydrological dimensions while adding to existing ecological, water quality, forestry, agronomic and Geographic Information System (GIS) skills. Social science would draw from and further develop existing anthropologists and teacher trainers and incorporate sensitivity to gender dynamics and other anthropogenic issues.

A watershed assessment study yields benefits in a hierarchy based on geographic/geomorphic scaling considerations (WEF, 1998). A key feature of watershed management is the use of models to understand the amount of pollution generated and the subsequent distribution and fate of pollutants throughout the watershed (Korfmacher, 2001). Typically scientists build watershed models that focus on bio-physical processes. However watershed management involves a greater reliance on social processes than other resource management foci. Therefore models should reflect that social process (Korfmacher, 2001; Paolisso and Maloney, 2001; Lazo et al., 1999). For example, "cultural models are presupposed, taken-for-granted models of the world that are widely shared (although not necessarily to the exclusion of other, alternative models) by members of a society and that play an enormous role in their understanding of that world and their behavior in it." (Paolisso and Maloney, 2001). Conceptual models serve as vehicles to build system understanding from qualitative data collection efforts (Soulliere et al., 2001).

Development projects can incorporate participatory approaches to watershed management (GEF, 2004; Wangila and Swallow, 2001; Kerr et al., 2000; Estrella and Gaventa, 1998). However, previous experience suggest various factors that influence success. The most effective efforts foster collaboration among local communities, national and international agencies, lay and techno-scientific knowledge. Financial and technical investments must exist complementarily and reflect local conditions to ensure sustainability. Efforts must identify, involve and incorporate diverse stakeholders using stakeholder appropriate policy solutions. Watershed efforts need to acknowledge heterogeneity within watersheds. While watersheds fit nicely into physical and hydrologic boundaries, watersheds rarely provide the appropriate social scale for management (Kerr et al., 2000). Lastly, communities with previous experience in community-based or cooperative efforts in other areas and without inconclusive or problematic property rights conditions, provide the best chance of success (Wangila and Swallow, 2001; Kerr et al., 2000).

Deliverables from the watershed as a whole include an inventory of resources, statement of environmental endpoints and quality targets, list of Best Management Practices (BMPs) and mitigating measures and location, list of impact statements and uncertainties to be addressed in future monitoring programs. From the social perspective, the center should be able to identify a meaningful set of indicators for measuring group activity related to key social goals and then track changes in social organization as part of project monitoring.

Research Design

The study will involved a detail review of literature – legal and policy documents, reports (published and unpublished – e.g., past project documents) and also involve reconnaissance field visits. The watershed will be broadly zoned based of survey map of Kenya to enable focused and targeted field visits to cover key system characteristics. An initial approach would be a visit to district headquarters and relevant government agencies to determine how much literature is available. The extent of field survey will then depend on available information. The component leader will liaise with overall project principal investigator to ensure that the component report is according to agreed standard for all project components.

Table 1. Steps in Completing a Watershed Assessment

Step	Item
1	Identifying and networking with local agencies and citizens (social inventory)
2	Getting to know the watershed (agronomic, hydrologic, river morphology, ecological inventories); Income sources, Watershed goals
3	Defining the critical areas, including stream and wetlands physical condition assessment
4	Surveying and inventorying key features (Mapping, field visits, etc.); identify reference streams for desired ecological, hydrological, and water quality conditions
5	Prioritizing pollutants, sources and causes
6	Determining objectives for further action (education, research) based on reference metrics
7	Identifying potential best management practices needed based on reference metrics
8	Identifying and analyzing existing local projects, programs and ordinances; promote a few self perpetuating practices
9	Informing and involving the public
10	Developing an evaluation process in light of watershed goals
11	Assembling the work plan

The present hydrological conditions of the watersheds are as a result of many processes and activities both anthropogenic and natural. Environmental assessment, stimulated by the US Clean Water Act, is now becoming a well developed science. To start a watershed assessment, one must first state the goal; determine a route to that goal, then work to accomplish the goal. Barbour et al. (1999) provides details on hydrologic and ecological assessment techniques.

The end goal of a watershed assessment effort is to comprehensively model water quantity and quality delivered to the discharge. Modeling, while not necessary to complete an assessment, enables a structured approach to planning an assessment process in that they specify the nature of the data needed. Available models then enable the watershed planner to evaluate management strategies to provide guidance for policy decisions.

Perhaps the single most important variable in achieving sustainable watershed management is understanding and underpinning key land use practices that directly or indirectly affect ecological processes and system functioning. The reliance on land for agrarian production in rural Kenya coupled with dependence on land resources for economic livelihood places enormous premium on resources derived from land and as a consequence leads to degradation and hence loss of ecological integrity of the system (GEF, 2004; Githaiga et al., 2003; Osana et al., 2003). A sustainable land management strategy requires not only intervention at site specific but also the landscape level. A system approach is needed to disentangle critical landscape components and linkages and will more likely to lead to overall positive impacts on the watershed. The Nzoia watershed system transcends a broad range of land use systems and practices ranging from small-scale holder farmland to large scale mechanized agriculture, and cuts a cross a tenure regime of private ownership to public land – e.g., forest reserves and national parks. The watershed produces 30% of Kenya's maize and sugar (Osana et al., 2003). The watershed occurs in generally high potential and high population region of the country and therefore the influence of land use on the system is extremely important (Osana et al. 2003). Eldoret serves as the largest population center (234,000) followed by Kitale (88,100), Kakamega (86,500), Webuye (45,100), Mumias (36,200) and Bungoma (32,900) (Osana et al., 2003). Although agriculture comprises the major land use, textile, paper, sugar and coffee processing comprise major point source pollutants (Osana et al., 2003).

A GIS-based framework will be established to manage the watershed descriptive data. The GIS approach is a layered data management approach (Haan et al., 1994). GIS enables one to identify selected parameters. DeBarry (2004) provides an exhaustive listing of possible GIS layers for watershed assessment. He also provides an orderly approach towards the development of a GIS database. Sayer et al. (2000) provide an excellent set of forms and tables for terrestrial ecological assessments. Canter (1996) is but one example of a compendium of techniques for assessing land and water resources. He provides matrix, network and checklist tools for impact identification related to surface water, soil and groundwater environments (Table 1, items 3, 4). Poor hydrologic conditions of watersheds are known to contribute greatly to the deterioration of watersheds particularly with respect to the water quality and quantity. It is necessary to understand the interrelationship of these processes in order to suggest interventions that will lead to the arresting of these conditions and possibly reversing them. River morphology methods such as the Rosgen (1996), while not developed in the same physiographic soil group, can nevertheless provide valuable indications as to what increased development in the watershed can do to the river. Similarly, these methods can provide valuable insights into how damaged areas can be rehabilitated. Because of the above facts, it is important to understand the various physical aspects of the watershed and their contribution to the overall hydrologic status i.e., its water quality and quantity conditions. By carrying out an in-depth inventory/characterization of the hydrological condition, it is possible to identify pressure points, which ultimately form the entry avenues for the interventions (Table 1, items 5, 6). The rehabilitation as a goal for most elements of the watershed presupposes knowledge of the current and reference conditions of both functioning and nonfunctioning watershed processes. Thus, both qualitative and quantitative data will be a prerequisite for the watershed rehabilitation. Mapping and initial feature identification will be completed in conjunction with the capacity building objective (GEF, 2004).

Regional Integration

The project is targeting the development of a watershed and basin assessment center at Moi University by building a physical science and social science interdisciplinary center to complement such centers at Egerton and Nairobi universities. This project is inherently integrative as it initiates and increases cooperation among diverse interests with the common thread of maintaining the health of a common benefit, the community river. The concept is replicable in other parts of Kenya as well as other developing countries. Close working collaboration will be maintained with the Sustainable Management of Watershed – CRSP (SUMAWA) project that studying the River Njoro watershed. The concept is replicable in other parts of Kenya as well as other tropical regions.

Schedule

Research Activity Plans	**Responsible Person(s)**	**Personnel**	**Timeline**
Conduct capacity needs assessment with regard to personnel and equipment	Muchiri	Odipo, Kiyiapi, Khamasi, Karanja,	March, 2005
Field reconnaissance visit and identification representative subwatersheds for detailed assessment	Muchiri	Tollner, Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	March, 2005
Conduct review of historical data on land uses, policy and tenure regimes	Kiyapi	Kinagwi, Mureithi	March, 2005
Begin review of historical data on hydrologic characteristics and conditions of the Nzoia River	Farah	1 graduate student	March, 2005
Begin conduction of a broad land use mapping of Nzoia basin	Kiyapi	Tollner, Karanja, Muchiri	March, 2005
Build relevant watershed assessment literature collection	Muchiri, Tollner, Habron	Khamasi, Odipo, Kiyiapi, Karanja	March, 2005
Development of key measurement capabilities and GIS integration	Tollner, Muchiri	Karanja, Odipo	March, 2005
Serious environmental impact zones	Muchiri, Tollner	Tollner, Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	December, 2005
Graduate students training at Moi University	Muchiri	Tollner, Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	April, 2006
Complete resource inventory	Muchiri, Tollner	Tollner, Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	April, 2006

Statement of key environmental endpoints and quality targets	Muchiri, Tollner	Tollner, Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	April, 2006
BMPs and mitigating measures for identified key locations	Muchiri, Tollner	Tollner, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	April, 2006
Key social indicators	Muchiri, Habron	Tollner, Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Muhia, Farah	April, 2006

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Land-use Practices, Policy and Tenure Regimes in the Nzoia River Basin

Environmental Impacts Analysis (11.5EIA5)/Study/Kenya

Investigators

James Kiyiapi	HC Principal Investigator	Department of Forestry, Moi University
Ruth Kinagwi	HC Principal Investigator	School of Environmental Sciences, Moi University
E. W. Tollner	US Principal Investigator	University of Georgia

Objectives

1) Develop digitized land use maps for the Nzoia River basin.
2) Conduct a broad landscape level characterization of land uses, practices and interactions.
3) Map broad land use zones.
4) Identify factors that drive land use changes.
5) Analyze and evaluate existing policies related to land use.
6) Determine influence of existing land policies and laws on land use practices.
7) Examine extent to which the existing legal and policy framework affects and protects the environmental integrity of the basin.
8) Assess extent of awareness of environmental/land policies and laws with regard to land use practices within the selected sub-watersheds.
9) Determine awareness or lack thereof of existing legislation.
10) Examine extent to which existing laws aid or impede land owners in their land use.
11) Determine community views on environmental matters and watershed protection.

Significance

Perhaps the single most important variable in achieving sustainable watershed management is understanding and underpinning key land use practices that directly or indirectly affect ecological processes and system functioning. The reliance on land for agrarian production in rural Kenya coupled with dependence on land resources for economic livelihood places enormous premium on resources derived from land and as a consequence leads to degradation and hence loss of ecological integrity of the system. A sustainable land management strategy requires not only intervention at site specific but also the landscape level. A system approach is needed to disentangle critical landscape components and linkages and will more likely to lead to overall positive impacts on the watershed. The Nzoia watershed system transcends a broad range of land use systems and practices ranging from small-scale holder farmland to large scale mechanized agriculture, and cuts a cross a tenure regime of private ownership to public land – e.g., forest reserves and national parks. The watershed occurs in generally high potential and high population region of the country and therefore the influence of land use on the system is extremely important.

Aside from the analysis of land use practices and associated possible environmental effects, another key aspect of this component is to examine the role and influences of prevailing policies and laws as drivers of land use practice. The extent to which the land use systems and practices are guided or influenced by existing laws and policies. An understanding of how much overlap or synergy exist between various land related policies and legislation and how these might impede sustainable land use management at the site specific level and overall watershed system. How much environmental /land use policy and legislative awareness exists amongst the rural population within the watershed? What policy or legal provision exist for mitigating against negative environmental impacts of land use practices e.g., use of pesticides and herbicides? These are some of the critical questions that need to be addressed in this component.

Anticipated Benefits

Overall, the land use characterization will offer a detailed typology of land and resource tenure interactions, as well as land use practice continuum (underpinning economic drivers and system constraints).

We anticipate that this work will lay the foundation for a systems approach to landscape management in Kenya.

Research Design

Location: All work on this study will be done in Kenya.

Methods: The study will involved a thorough review of literature – legal and policy documents, reports (published and unpublished – e.g., past project documents) and also involve field visits. The watershed will be broadly zoned based on survey maps of Kenya to enable focused and targeted field visits to cover key system characteristics. An initial approach will be visits to district headquarters and relevant government agencies to determine how much literature is available. The extent of field survey will then depend on available information. The component research team will refine and draw clear and achievable objectives, tasks and outputs and timeframe.

Conduct review of historical data on land uses, policy and tenure regimes: This will involve a detailed review of published and the so-called grey literature (in form public documents, available project reports, district development plans, information available on the internet). The analysis will specifically focus on:

1) An historical evolution of land tenure in Kenya with specific reference to Nzoia River basin
2) Underpin the push-and-pull factors responsible for current land use situation.
3) Examine the extent to which government policy (formal or informal) has influenced tenural rights and given rise to present land use situation.
4) Examine critically the extent to which the prevailing legal and policy framework affects use and protection of the watershed.

Link results from the review to the ongoing national land policy formulation: this will involve holding consultation with senior officials of the Ministry of Lands, Settlement and Housing, and also consulting with any NGOS and/or CBOs working in the area on aspects related to land.

This synthesis will focus primarily on land tenure evolution in relation to land use and natural resource management rather than on the broader issue of property rights. This component of the study will take no more than 1-1.5 months to obtain literature and compile analysis.

Conduct a broad land use mapping of the whole Nzoia basin: The work will involve some rapid transect drive across the basin covering upper, mid and lower stream zones. In order for the broad mapping to be done: three stages are envisaged:

Use existing satellite images and topo sheets to identify key land use zones (agricultural/settlement zones, major public installations, key protected areas – forests and research farms. Identify land use zones or areas where data from the satellite or topo sheets are unclear and for which GPS points will be taken. The whole basin will be digitized from satellite and topo sheets and this will provide basis for updating the maps.

Rapid assessment techniques will be used to map out major land use zones: agricultural zone, river basin area experiencing high intensity of use, highly degraded areas within the basin, grazing land, protected areas etc that require closer attention. The rapid assessment will entail driving across and along the river basin and through consultation with government agencies, local communities, and other agencies.

Conduct a survey to determine extent of awareness of environmental /land policies and laws awareness with regard to land use practices within sub-watersheds: Under this component of the land use research two key activities will be undertaken:

1) Conduct a detailed review and analysis of the relevant Acts of Parliament that have direct bearing on land use and natural resource management. This includes the Land Adjudication Act, Water Act, Agriculture Act, Forestry Act, Fisheries Act, Chief's Authority Act and the newly

enacted Environmental Management and Coordination Act (EMCA). Analyze extent to which EMCA has provided a framework for environmental protection and conservation vis-à-vis provisions of the other Acts. This review focus on how the existing land and natural resources laws respond to and enhance:

- Environmental (especially watersheds) protection?
- Community participation in land management and environmental protection
- Creation of viable local level institutions (registered community environmental groups, mechanism for inter-sectoral collaboration among various government and non-governmental agencies
- Enforcement of existing legislation and capacity of enforcing agencies

2) Conduct a preliminary sampling survey to assess awareness of above pieces of legislation among the local population. A questionnaire will be designed to obtain responses and probe aspects such as:
    - Dominant land use type
    - Environmental /ecological constraints encountered by local people (lack of water, low crop yields)
    - Extension services provided by government departments or other service agencies
    - How existing laws aid or impede land owners in use of land
    - Awareness or lack thereof of existing legislation
    - Encounters or experiences with environmental law enforcement agencies
    - General community views on environmental matters and watershed protection

The questions will be constructed to elicit quick and direct responses. To ensure effective use of field time and build synergy, random interviews will be conducted among land owners and other stakeholders within selected sites or localities. In addition to random survey targeting a minimum sample size of 50 respondents, focused group discussions will also be held to obtain general community /land owners dynamics. The questionnaire sampling will be as representative as possible of the different layers of society (across various age strata, gender and socio-economic groups). This survey is expected to provide a good level of stratified data.

Statistical Design, Null Hypothesis and Statistical Analyses:
Surveys will be designed using appropriate stratification techniques. The null hypothesis is that there are no differences across age, gender and socioeconomic groups. Data will be analyzed using commonly available techniques such as contingency tables, frequency tables, correspondence analyses and log-linear models (Hintze, 2004).

Regional Integration

Close working collaboration will be maintained with the Sustainable Management of Watershed – CRSP (SUMAWA) project that studying the River Njoro watershed. Through Dr. Geoffrey Habron, a link will be established with the Land-Use Change Impacts and Dynamics (LUCID) project.

Schedule

Conduct review of historical data on land uses, policy and tenure regimes (January 2005–June 2005);
Rapid Assessment to map out major land uses (June 2005–September 2005);
Collect data to determine extent of awareness on environmental and land policies and laws in two identified sub-watersheds (June 2005–September 2005);
Data analyses and reporting (October 2005–December 2005).

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Workshops on Guidelines for Developing Aquaculture Best Management Practices

Environment Impacts Analysis 6 (11.5EIA6)/ Activity/Brazil, South Africa

Investigators

Claude E. Boyd	US Principal Investigator	Auburn University
Chhorn Lim	HC Principal Investigator	Auburn University
Lúcia H. Sipaúba Tavares	HC Principal Investigator	Universidade Estadual Paulista, Jaboticobal, São Paulo, Brazil
Julio F. Queiroz	HC Principal Investigator	Embrapa Environment, Jaguariúna, São Paulo, Brazil
Khalid Salie	HC Principal Investigator	Stellenbosch University, South Africa
Lourens de Wet	HC Principal Investigator	Stellenbosch University, South Africa

Objectives

1) Discuss the general environmental issues affecting aquaculture.
2) Discuss the purpose of codes of conduct and best management practices with examples of their use.
3) Present guidelines for developing best management practices for aquaculture.
4) Prepare a manual on developing best management practices for aquaculture.

Significance

Sustainability and environmental protection are major issues in aquaculture (Black, 2001). Unsustainable projects are economically damaging, but often, they are the aquaculture endeavors that do the most environmental harm (Clay, 2004). Two main reasons for some aquaculture projects failure to achieve economic and environmental sustainability are selection of inferior sites (Hajek and Boyd, 1994) and application of inappropriate management procedures (Boyd and Tucker, 1998).

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 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 began 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, 2003 a, 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 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 (Avault, 1996) and there is much interest in developing a significant aquaculture industry in South Africa (Annonymous, 2004). Workshops to present procedures and process that should be followed in developing aquaculture BMPs will be valuable to future efforts to improve the environmental 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. The manual initially will be prepared in English. It will be translated to Portuguese and African by the host country PIs. The English version possibly can be presented as a Aquaculture CRSP publication. If additional funding can be found by the host country institutions, the translations also will be published. Moreover, it may be possible to later present the workshop on BMP development in other nations where Aquaculture CRSP has activities.

Anticipated Benefits

The overall benefit expected from these workshops will be to present the process that should be followed in developing science-based, aquaculture BMPs through a transparent, consultation involving a wide range of stakeholders. The specific benefits expected to accrue from this activity are as follows:

1) A workshop on development of aquaculture BMPs will be presented to stakeholders in Brazil and South Africa.
2) The need for following an acceptable process in developing aquaculture BMPs will be promoted.
3) A manual to guide those desiring to develop aquaculture BMPs will be written.

Workshop Design or Activity Plan

Participants: It is anticipated that about 50 people will attend the workshop in each country. The stakeholders will be fish farmers, feed producers, aquaculture researchers, extension workers, and administrators, scientists from governmental environmental agencies, representatives of environmental NGOs, and media specialists. In addition, aquaculture, fisheries, and environmental science graduate students from host universities will be urged to participate.

Location: The workshops will be held at the Aquaculture Center at São Paulo State University in Jaboticobal, SP, Brazil and at the Aquaculture Unit of the Faculty of Agricultural Sciences, Stellenbosch University, Stellenbosch, South Africa. Both are well known and respected by local and national aquaculture industries. Moreover, the two institutions have adequate facilities for convening the workshops and the locations can be easily reached by travelers.

Workshop Plan and Methodology: The workshop will be held in South Africa in October or November 2005 and in Brazil in January or February 2006. Each workshop will be 2 days in length.

Participants will be required to cover their own expenses. Preliminary contacts by the host country PIs suggest that this requirement will be acceptable and adequate attendance can be achieved. In Brazil, the workshop will be translated from English to Portuguese by Dr. Julio Queiroz who has much experience in research on aquaculture and the environment.

The tentative outline for the workshop follows:

Day 1

9:00-9:30	Introduction
9:30-10:30	Aquaculture and the Environment
10:30-11:00	Break
11:00-12:00	Codes of Conduct and BMPs: An Introduction
12:00-1:30	Lunch
1:30-3:30	Presentations about National Aquaculture

	• Production statistics and future trends
	• Market considerations
	• Culture systems
3:30-4:00	Break
4:00-5:30	• Environmental impacts
	• Aquaculturist's opinion
	• NGO concerns
	• Aquaculture regulations

Day 2

8:30-9:15	Environmental Impact Assessment in Aquaculture
9:15-10:00	Site Evaluation Issues
10:00-10:30	Break
10:30-11:00	Developing Draft BMPs to Prevent or Mitigate Impacts
11:00-12:30	Involving Stakeholders in Review and Refinement of BMPS
12:30-1:30	Lunch
1:30-3:00	Guidelines for Implementation of BMPs
3:00-3:30	Break
3:30-4:00	Application of BMPs
4:00-6:00	Questions and Answers

One of the US PIs (Boyd) will make the presentation on aquaculture and the environment and introduce the topic of codes of conduct and BMPs. National speakers will be identified to present information on aquaculture in each country. On the second day, the US and host country PIs will be responsible for presenting information on the process of BMP development.

Regional Integration

The effort has great prospects for regional integration in all Aquaculture CRSP regions because there is widespread interest in aquaculture BMPs. The manual could be used widely by those interested in developing BMPs. Also, interest and funds permitting, the workshop could easily be presented in other nations.

Schedule

The tentative schedule follows:

South Africa

February-September 2005	Planning for workshop
October 2005	Present workshop
October 2005-April 2006	Follow up on questions; finalize manual (US PIs will be the leaders for this task)

Brazil

February 2005-January 2006	Planning for workshop
February 2006	Present workshop
March-April 2006	Follow up on questions; finalize manual (US PIs will be the leaders for this task)

30 April 2006: Termination of project (and report submission).

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Amazon Aquaculture Outreach

Sustainable Development and Food Security 1 (11.5SDF1)/Activity/Peru, Bolivia, Brazil, Colombia, Ecuador

Investigators

Christopher C. Kohler	Project Director and PI	Southern Illinois University at Carbondale
Susan T. Kohler	Co-PI	Southern Illinois University at Carbondale
William N. Camargo	Project Coordinator	Southern Illinois University at Carbondale
Salvador Tello	HC Prime Site Project Leader	Institute for the Investigation of the Peruvian Amazon, Peru
Fernando Alcántara	Host Country Prime Site PI	Institute for the Investigation of the Peruvian Amazon, Peru
Mariano Rebaza	Host Country Prime Site Co-PI	Institute for the Investigation of the Peruvian Amazon, Peru
Marina Del Aguila	Host Country Co-PI	Universidad Nacional de la Amazonia Peruana
Guillermo Alvarez	Host Country Collaborator	Fondo Nacional del Desarrollo Pesquero (FONDEPES), Peru
Mabel Maldonado	Host Country Collaborator	Universidad Mayor de San Simón, Bolivia
Mabel Magariños	Host Country Collaborator	Universidad Mayor de San Simón, Bolivia
Amalia Antezana	Host Country Collaborator	Universidad Mayor de San Simón, Bolivia
Marle A. Villacorta C.	Host Country Collaborator	Universidad Federal do Amazonia, Brazil
Rodrigo Roubach	Host Country Collaborator	Instituto Nacional de Pesquisas da Amazonia (INPA), Brazil
Levy Caraballo		EMBRAPA, Manaus, Brazil
Edwin Agudelo	Host Country Collaborator	Instituto de Investigaciones de la Amazonía SINCHI, Colombia
Santiago Duque	Host Country PI	Instituto de Investigaciones IMANI, Leticia, Colombia
Carlos Augusto Pinto	Host Country Collaborator	Instituto de Investigaciones IMANI, Leticia, Colombia
Suzana Ricaurte	Host Country Collaborator	Peace Corps, Ecuador
José Machoa	Host Country Collaborator	Comunidad Indígena Sarayaku, Ecuador
Luis Arevalo A.	Host Country Collaborator	Fundación Arcoiris, Ecuador
Ricardo Burgos M.	Host Country Collaborator	Fundación Arcoiris, Ecuador
Galo Plaza M.	Host Country Collaborator	Instituto Tecnológico Saleciano, Ecuador

Objectives

1) Provide extension services to the community to promote sustainable aquaculture in the Amazonian region.
2) Provide short courses to governmental and NGO personnel to maintain and expand a network of aquaculture extensionists in the Amazon region.
3) Maintain the specialized website developed in the Tenth Work Plan on Amazonian aquaculture to provide for information exchange and networking.

Significance

Fish culture has been practiced for over five decades in the Amazon region. The countries comprising the Amazon region are linked by major river systems, particularly the drainages comprising the Amazon and Orinoco Rivers. The largest diversity of freshwater fishes in the world is contained within these drainages.

In the Peruvian Amazon, the food security program (PROSEAL) created in 1999 and directed by Terra Nouva (Italian NGO, Marco Colace) and IIAP, provided significant extension service (five extension agents) for several communities along the Iquitos-Nauta Road and the Santa Helena and Huayococha indigenous communities in Tigre River (Maynas Province, Loreto Department, Peru). After Terra Nouva completed its prime goals in December 2001, CRSP and IIAP took over this important task through a transitional period in 2001 by rehiring up to 2 of the 5 extension agents, and by enrolling a third extension agent in a Ph.D. program at SIUC. Results from research conducted at our host country facilities provided much of the information that PROSEAL extended to farmers. Thus, at the outset in 1999, PROSEAL was a direct beneficiary from the CRSP program in Peru. As a result of the technical support and outreach efforts of the CRSP/IIAP team in the Ninth, Tenth, and Eleventh Work Plans, valuable information has been transferred to the Iquitos and Tigre River area fish farmers.

In the Amazon Department (Colombia) a new aquaculture association (Acuiamazonas) was integrated during a visit by one of our Peruvian extensionists (Carlos Chavez). Acuiamazonas has more than 50 members, who live along the Leticia-Tarapacá Road. Additionally, several indigenous groups also inhabit this road and they have demonstrated interest in aquaculture related activities as part of a food security program.

In Brazil, a project under the direction of Prof. Marle Villacorta (Universidad Federal Do Amazonas) with the financial support of the Brazilian government titled: "Family fish culture and food processing in the Sateré-Mawé indigenous communities along the Marau and Urupadi Rivers" is underway. The main objective is to implement ethnodevelopment activities of the Sateré-Mawé indigenous communities (32 communities with 4,160 inhabitants) to guarantee food security. The Sateré-Mawé indigenous community inhabits the area between the Amazonas and Pará States (middle region of the Amazon River). Fishing is the main source of animal protein for the Sateré-Mawé, but the Marau River is overfished and aquaculture is seen as a means to ensure food security for the rural poor.

Anticipated Benefits

The development of sustainable aquaculture will benefit many sectors throughout the Amazon region. Rural farmers and indigenous communities will benefit from the addition of an alternative form of agriculture. Aquaculture production requires considerably less land than that needed for cattle ranching. Moreover, ponds can be used year-after-year whereas rain forest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons. Such lands, once abandoned, usually can no longer support normal jungle growth. Both rural and urban poor will benefit by the addition of a steady supply of high quality protein in the marketplace. Aquaculture of Colossoma 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; Chu, dissertation in progress). Accordingly, the aquaculture of Colossoma and Piaractus may be ecologically as well as economically and nutritionally beneficial to the inhabitants of the 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 will benefit from this activity. Development of a Latin American network of Amazonian species producers and researchers has begun to catalyze regional efforts to fortify the growing industry and to explore new aquaculture candidates to diversify production in this highly productive and species-rich region. Specifically, we will quantify the following:

1) Number of fish farmers receiving extension services.
2) Number of training participants (extension agents, students and experts from Amazon region).
3) Number of hits at Amazonian aquaculture web page.

Activity Plan

Location: This work will be conducted and/or directly impact Bolivia, Brazil, Colombia, Ecuador and Peru.

Methods:

Objective 1. Provide Extension Services to the Local Community to Promote Sustainable Aquaculture in the Amazon Region

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

1) Provide workshops to existing and prospective fish farmers in the Iquitos region. Specifically, we will update the Spanish-language production manual for Colossoma and Piaractus compiled by the Tenth and Eleventh Work Plans to accompany the reproduction manual completed in the Ninth Work Plan. These companion manuals will be used in workshops to be conducted at the IIAP Quistococha Aquaculture Station and at Aquarios Leticia in Leticia (Colombia) for teaching prospective farmers the basics for pond culture. One workshop will be provided in Work Plan 11.5 to prospective fish farmers in the region in high schools and technical (vocational) schools. The workshop will also include orientation on the business aspects of aquaculture.

2) Provide aquaculture advisement via site visits to local farmers. We will continue to make bimonthly site visits to fish farms in the Loreto Department (Peru) and Amazonas Department (Colombia). Farms will be visited on a rotational basis so that every farm is visited at least once each quarter. Farmers will be provided with information on fish husbandry and pond maintenance, as well as with any new developments learned through our research activities. Standard water quality parameters (temperature, dissolved oxygen, pH, total ammonia nitrogen, and nitrite) will be measured at representative farms throughout the region whenever required (i.e. massive fish mortalities, etc.

3) Evaluate the extension service through a questionnaire pilot tested and administered by the extensionists themselves to all clientele receiving extension services to assess quality of extension provided and to obtain suggestions on how to improve the program.

Objective 2. Provide a Short Course to Governmental and NGO Personnel to Develop a Network of Aquaculture Extensionists in the Amazon Region

One intensive training course for a small group of governmental and non-governmental personnel conducting aquaculture research and/or extension activities in the Amazon Basin will be offered at Universidad Federal do Amazonas, Manaus, Brazil. This training plan will continue with the very successful program that has so far trained over 100 extensionists and fish producers from Bolivia, Brazil, Colombia, Ecuador and Peru. For the course, six qualified candidates from Amazon countries will be invited to participate. The course will be offered to train in extension techniques a designated representative from several indigenous communities, as well as aquaculturists and experts in aquaculture related areas. Techniques which have been practiced successfully by IIAP and Terra Nuova, including CRSP's new experiences in the region through the Eleventh Work Plan, will be taught. Extension personnel will also learn pond construction, broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, disease prevention and treatment, all specifically related to native cultured species of *Colossoma* sp., *Piaractus* sp., *Arapaima* sp., *Prochilodus* sp. and *Brycon* sp. (fish), and Congompe and Churo (mollusks). A CD-ROM displaying all the course material for the Amazon aquaculture-training course will also be produced to complement the written manuals.

Objective 3. Maintain and Expand the Specialized Website on Amazonian Aquaculture and Species to Provide for Information Exchange and Networking

A website (http://ws1.coopfish.siu.edu/amazonia/index.html) on Amazonian aquaculture and species will be maintained and expanded to allow for information exchange and networking. The web site will contain information on all CRSP-sponsored research and outreach activities in the Amazon region. It will also provide links to other agency activities in the region such as USAID, World Wildlife Fund, etc. An "AquaForum" will allow for discussions on Amazonian aquaculture and species by interested participants. The website will contain a specialized bibliography on publications on research and outreach activities related to Amazonian aquaculture and species. An up-to-date list of announcements concerning related workshops and meetings will be maintained on the site. A listserve will be established and maintained for the purpose of relaying relevant information on Amazonian aquaculture and species. The number of hits to the site will be enumerated to determine the site's exposure.

Regional and Global Integration

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

Schedule

All activities will take place from 1 August 2004 through 30 March 2006. The final report will be submitted by 31 May 2006.

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Understanding the Aquacultural Knowledge and Information System for Commercial Tilapia Production in Nicaragua: Economics, Institutions, and Markets

Sustainable Development and Food Security 2 (11.5SDF2)/Study/Nicaragua

Investigators

Joseph J. Molnar	US Principal Investigator	Auburn University
Daniel E. Meyer	HC Principal Investigator	Panamerican Agriculture School, Zamorano, Honduras
Curtis Jolly	Collaborator	Auburn University
Greg Traxler	Collaborator	Auburn University

Objectives

1) Provide a baseline perspective on the state of tilapia aquaculture in Nicaragua.
2) Describe the Aquacultural Knowledge and Information System for tilapia culture in Nicaragua.
3) Conduct case studies of functioning clusters of medium-scale commercial producers.
4) Identify the barriers and constraints to commercial aquacultural development in Nicaragua.

Significance

An assessment of Nicaragua's growing aquaculture sector identified tilapia as one of a series of and opportunities to strengthen exports and business investment (USDA-FAS, 1995b). The Central American Free Trade Agree favors non-traditional exports such as tilapia, shrimps, oriental vegetables, fruit, melons and tobacco (USAID, 2003a, 2003b). Thus tilapia is identified as focal activity for developing the Nicaraguan economy. Norwegian interests are active in marine farming in Nicaragua. A Norwegian firm is the principal shareholder in Nicanor, the largest fishery group in Nicaragua. Nicanor built a modern value-added processing company and is investing in aquaculture equipment for tilapia in 2002.

An Aquaculture Knowledge and Information System (AKIS) is a system of people and institutions that generates, transfers, and utilizes aquacultural knowledge and information (Berdegu and Escobar, 2001, FAO and World Bank, 2000). The AKIS for tilapia comprises the private firms, nongovernmental organizations (NGOs), and public agencies involved in the generation and modification of knowledge, and in the transmission and exchange of information, relating to aquaculture (Byerlee, 2004). The system is characterized by its key subsystems: research, extension, and education. Farmers -- their needs and opportunities -- drive education, extension, and research. Farmers should, but often do not, provide direct input into design, funding, priority setting, execution, and evaluation of these key subsystems (Van den ban and Hawkins, 1996).

Although the Nicaragua's AKIS may not be well articulated, the organizations, agencies, and firms working in aquaculture have different capacities and advantages, and play different roles (FAS, 1995). Once these are understood, their responsibilities and objectives may be better coordinated to avoid overlap and achieve complementarities. The central actors in most developing-country AKIS are farmers' organizations, farmers, and (government) research and extension organizations (Jensen et al., 1995).

In some AKIS, organizations such as NGOs and commercial enterprises may be central to technology generation and dissemination, and in all systems such organizations are active in technology and information flow to some extent (Eponou and Peterson, 1999). It is not clear how the cluster of firms, agencies, and organizations is functioning in Nicaragua or how this is contributing to aquacultural development (Porter, 2000, Schmitz, 1997). Efforts to expand aquacultural development are predicated on an AKIS that can stimulate producers and respond to their information and technical needs (Clark, 2003, 2004, Cash et al., 2003).

Investments in AKIS are of increasing importance to address needs of rural people and assure future food security and environmental sustainability. Nicaragua is a growing part of the global supply chain for shrimp. Tilapia may play an important role as rotational crop for the industrialized sector and as a viable enterprise for commercial producers oriented toward domestic markets (USDA-FAS, 1995a).

Hughes (2000) identified three broad areas with high potential for tilapia production in Nicaragua where farms and infrastructure already exist – Esteli-Matagalpa and the Central Highlands, Leon-Chinandega, and the coastal lowlands of both oceans.

In the coastal lowlands, industrialized shrimp and tilapia farms are a central aspect of the Nicaraguan AKIS. Nicaragua has the most area of land suitable for commercial shrimp farming in Central America with estimates exceeding 30,000 ha. More than 5 million pounds of farm-raised shrimp were processed in Nicaragua in 2004. There are at least four plants that process farm-raised shrimp. Some purchase from producers and others custom process under farm brand labels (USDA-FAS 1995a). As tilapia is often employed as a rotational crop for shrimp, this has important implications for the development of the infrastructure for tilapia production.

Environmental organizations are a central part of the AKIS in Nicaragua as they are important voices in the dialogue shaping the regulation of commercial aquaculture. The first species of tilapia was introduced to Nicaragua from El Salvador in 1959. Current environmental concerns focus on industrialized production of tilapia in freshwater lakes (Gutierrez, 2001, Guillen, 2003).[1] Nicanor SA recently opened a tilapia farm and processing plant on the shores of San Ramon, Ometepe. Effluents have threatened Lake Cocibolca and environmentalists fear that continuous growing of tilapia would cause sedimentation problems, as occurred in Lake Managua. Molina (2003) asserts that the discharge from tilapia farms would be comparable to the untreated water from a city of 83 thousand people.

Away from the lakes, tilapia farming continues to be a viable, albeit less controversial, enterprise. In Las Chinas community, a USAID tilapia culture demonstration project was initiated in response to the coffee crisis.[2] Participating families have tilapia for their own consumption and for local market sales. The farmers received financing and technical assistance in setting up the ponds and raising tilapia (USAID, 2003a).

Much of the tilapia supplied to markets in Nicaragua is caught wild from lakes and reservoirs. Larger tilapia are processed as frozen fillets and either exported to the United States or sold to Nicaraguan supermarkets. Engle et al. (2001, 2003a. 2003b) conducted surveys of restaurants, supermarkets, and fish markets to show that tilapia are well known in Nicaragua. Wild-caught tilapia was sold by a majority of fish market vendors, but 26% of supermarkets and 21% of restaurants sold tilapia. Fish markets and supermarkets in Nicaragua do not appear to be viable market outlets because wholesale tilapia prices appear to be too low for farm-raised products.

Neira and Engle (2001) report that rural farmers need to enter into the marketplace with appropriate products in order to obtain adequate returns on investment and sweat equity. They conclude that the most promising markets for small-scale tilapia farmers appear to be restaurants and supermarkets because these outlets pay higher prices for fish than vendors in open-air markets.

The challenge to tilapia farmers is to raise the size of tilapia required to produce the preferred sizes of fillets. To produce the size fillet preferred by restaurants (87 g) will require farms to produce 580 g tilapia. The even larger size of fillet preferred by supermarkets makes this outlet even more challenging. Engle and Neira (2003) found that one-fourth of supermarkets in the country sold farm-raised tilapia in spite of the substantial fishery for tilapia in Lake Nicaragua. Tilapia was not sold due to off-flavor, lack of supply, and fears of selling contaminated fish from Lake Nicaragua. Tilapia farms and processors in Nicaragua will need to guarantee and ensure a consistent supply of good-flavor, high-quality, and safe tilapia products. The AKIS must work in a coherent way to achieve these objectives in the tilapia industry.

<u>**Anticipated Benefits**</u>

Direct target groups of the study will be:

1) The baseline assessment will provide an overview of the production capacity, infrastructure, and location of tilapia production in Nicaragua.
2) The AKIS in Nicaragua will benefit from a comprehensive assessment of the information and

market resources available for tilapia growing in that nation.

3) A minimum of 50 producers (men and women) will directly benefit from the farmer meetings and training that will take place in conjunction with the study. These fish farmers will be informed of the results of the study and receive our recommendations as to how to improve their production protocols/techniques and the quality of the fish they market.

Indirect target groups will be:

1) Approximately 2000 small-scale tilapia farmers will benefit by having better technical services available through training programs made available to NGOs.
2) The study will provide an opportunity for a graduate student to learn about the situation of local fish farmers and disseminate this information to other students at this institution and at technical meetings in Central America.
3) The study will enhance institutional understanding of the status of tilapia culture and the AKIS in Nicaragua, as well in two specific locales where medium-scale tilapia culture is progressing or has a high potential to progress.
4) Information and materials derived from this study will be useful for aquaculture development activities in other countries of Central and South America.

Research Design

Location: The fieldwork for this activity will involve multiple sources of information and perspective. The study is based on a series of interviews with public-sector decision-makers, representatives of fish culture organizations, ecological organizations, and nine discussion groups, each with a different profile in different areas where tilapia culture is expanding or has high potential to expand. The farmers are chosen on the basis of various criteria, primarily the size of their operation, age, expectations with regard to succession, and intensity of tilapia culture practices. These socio-economic and structural factors are emphasized in the literature as affecting implementation of aquaculture policies and support services.

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

Baseline perspective: We will seek documents and recent surveys of the aquaculture sector from knowledgeable individuals in Nicaraguan public agencies and private organizations. A synthesis of this information will be presented as an extended section or chapter in the project report. Additional information will emerge from the individual and group interviews.

Identification of study locales: Information will be obtained about national statistical sources about the regional distribution of tilapia farming and production. Based on interviews with industry leaders and responsible officials in aquaculture planning and management, two locales will be identified for more intensive study and the conduct of focused group interviews with tilapia producers. The Esteli-Matagalpa and the Central Highlands, Leon-Chinandega, and the coastal lowlands of both oceans have been noted as areas of high potential (Hughes 2000).

Conduct of Individual and Group Interviews: The design of this study follows the data triangulation research strategy described by Yin (1989). Key informant interviews will follow a positional and network sampling approach to conduct open-ended conversations with knowledgeable officials from public agencies, NGOs, and industry groups. In each locale, three sets of producer discussion groups of 8-12 participants will address marketing, production, and seed supply issues.

One set of discussion groups will be with established commercial tilapia farmers; a second group is with young farmers with recent experience in tilapia culture; and a third group is comprised of small producers with experience in tilapia culture primarily for home consumption or marketing at the village level. The discussion groups will address strengths, weakness, opportunities, and threats to tilapia production with each category of farmers. Thus, the case study results will summarize primary data from six focus groups and 12-18 interviews with knowledgeable individuals on the national, regional, and local levels.

Analysis: An Auburn University graduate student and a Nicaraguan graduate student will conduct the focus group interviews and collaboratively summarize the comments and insights expressed by participating tilapia producers. The report of this research is termed a descriptive case study, that is it will primarily describe what is happening and why, to show what the situation for tilapia culture in Nicaragua is like. The results may be especially useful to help interpret other data that may be available in subsequent research, such as survey data. The portrayal of the country situation as an AKIS also may be useful for project planners and government officials who can design research and extension programs in ways that more effectively utilize the organizational and institutional resources working in tilapia culture.

Regional and Global Integration

We plan a formal integration of this activity with the overall region. The work will be jointly conducted by Zamorano personnel (faculty, staff and students). In-country personnel (Suyapa Triminio) will assist in organizing training sessions in selected Central American Countries. Much progress has been made with the development of Web-based materials. Personnel at Zamorano have developed excellent interest and capability in serving as a web host. A cornerstone of our activity will build on experiences learned as we conclude the present work plan with training exercises in Guatemala, El Salvador, and Nicaragua.

A Spanish-language leaflet will make the central findings of the study available to the Nicaraguan AKIS.

Schedule

Desk study and review of the literature will take place during the first 6 months of the project
The intervening period will be devoted to fieldwork and analysis of the data.

The last six months of the project will be devoted to completion of the dissertation, and preparation and submission of the final report and summary leaflet in June 2006. We will endeavor to publish results in periodicals read by producers in Nicaragua, as well as serials read by scientists and instructors.

The survey and study of fingerling producers and inventory of NGOs will be realized during the period August 2003–June 2005.

As was done for the previous work plan, Quarterly progress reports will be submitted and a final report will be submitted at the end of the second year (June 2006). A report, spanning the two years of this activity is planned to be submitted to the Aquaculture CRSP by 30 June 2006.

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Endnotes

[1] The aboriginal name for this lake, Cocibolca (Nicaragua) means “sweet sea.” It is the largest lake in Central America and one of the very few, or perhaps the only, freshwater lake to have sharks, although their numbers have dropped precipitously. Cocibolca is one of the 40 largest lakes in the world by both surface area and volume. Its origins are both tectonic and volcanic.

[2] In the late 1990s, Brazil and Vietnam began flooding the market with cheap beans, causing a nearly 5-year-old global coffee crisis that forced scores of Central American Arabica-bean farms to shut down and destroyed the livelihoods of thousands of growers and workers. The glut sent prices plummeting on world commodities markets: Coffee selling for nearly $2 a pound wholesale in 1997 sold for less than 50 cents in 2001 and 2002. Small-scale farmers and those who produced lower-quality Arabica beans faced little or no demand for their crops. In Central America, farmers and pickers fled to cities in search of food and jobs. Over the past year, prices have recovered somewhat on the exchanges. Still, some growers struggle to make a profit and must weigh whether to abandon coffee. Tilapia is an alternative enterprise for some of these growers.

Assessing the Potential for Aquacultural Development to Promote Food Security Among Indigenous People in Guatemala

Sustainable Development and Food Security 3 (11.5SDF3)/Study/Guatemala

Investigators

Joseph J. Molnar	US Principal Investigator	Auburn University
Daniel E. Meyer	HC Principal Investigator	Panamerican Agriculture School, Zamorano, Honduras

Objectives

1) Improve understanding of the how aquaculture contributes to the livelihoods of indigenous peoples and other vulnerable groups with the intent of designing or reorienting fish culture technical assistance and research to better meet food security needs and livelihoods priorities.
2) Assess human organizational and institutional capacities in indigenous rural communities and the effectiveness of participatory community based approaches to aquacultural development in responding to the needs and reducing the food insecurity of the poorest and most disadvantaged groups.
3) Assess the nongovernmental organizational capacity and training needs to deliver technical assistance in tilapia culture to indigenous peoples and other vulnerable groups.

Significance

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. 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.[1] 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 pluricultural society in which 55% of its 14 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 (Stavenhagen, 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, 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.[2] Specifically, water temperature shapes the viability of tilapia as a farm enterprise. In the highlands the daytime air temperatures average between 20 and 25C all year long, but can fall below 10C 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 degrees might be considered an initial target category of communities for investigation. These roughly correspond to the coffee-growing belt in mountain areas that is less than 2000 m in elevation. Our study would endeavor to profile the needs and possibilities for raising tilapia among indigenous people in areas with appropriate technical conditions for fish culture.

In Guatemala, the total of freshwater ponds just exceeds 100 ha, which is less than 10% 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. 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 hectare per household and an average total annual income of US$700. By 1989, 1,200 ponds had been built or renovated. About 15% of these ponds were integrated with animals and 21% 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).

In Guatemala, aquaculture extension is provided by the Fisheries and Aquaculture Directorate, formerly by US Peace Corps, CARE, and by a Chinese technical mission. 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 the smaller farmers (Noriega-Curtis and Rivas, 1989).

Our main source of colleagues 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).

Anticipated Benefits

Direct target groups of the study will be:

1) Two indigenous communities with appropriate water availability and temperatures.
2) Five NGO technical assistance providers will be informed by the insights and understandings about the technical assistance needs and organizational mechanisms associated with successful small and medium scale adoption of tilapia culture.
3) A minimum of 50 individuals (fish farmers and extension agents from NGOs and government agencies) will receive technical training in the areas of brood-stock selection and management, tilapia reproduction and production and distribution of fingerlings.

Indirect target groups will be:

1) We expect that more than 20 indigenous communities will benefit by having better quality technical services and information to guide decisions about tilapia adoption and actual implementation of the enterprise
2) A minimum of 50 individuals from Central America will be trained in the selection and management of tilapia brood-stock, and in techniques for tilapia reproduction and fingerling production.
3) These activities will enhance Zamorano´s and Auburn's institutional understanding of the status of small-scale tilapia culture in the Latin American region and our abilities to propose and undertake effective development projects with aquaculture components.

The information and training materials derived from this study will be useful for aquaculture development activities in other countries of Central and South America where indigenous communities could benefit from the adoption of tilapia culture.

Research Design

Location: The fieldwork for this activity will occur in two purposively-selected, representative communities in different Guatemala departments. The worst indices of extreme poverty and absence of social services (human development indicators) are the departments of Totonicapan, Huehuetenango, Quetzeltenango and San Carlos where 70 per cent of the population are indigenous (Stavenhagen, 2002). Guided by colleagues from San Carlos University and the staff of NGOs working in target departments we will conduct participatory exercises, intensive interviews, and other social science data collection procedures to understand interest, barriers, and appropriate intervention points for aquacultural development in indigenous communities.

Methods:

Identification of Target Communities: We plan to make extended field visits of approximately ten days each to two communities, in different Guatemala departments. We will use physical maps and the expertise of aquaculture professionals and NGO personnel working with indigenous communities to identify locales that show interest and have good technical potential for successful conduct of fish culture. We will rely on the experience and expertise of NGO personnel to gain access to indigenous communities and to arrange translation where Spanish is not spoken. We seek communities that have experience with tilapia culture through the efforts of the Peace Corps or NGOs but now do not practice fish culture, communities where tilapia ponds are currently functioning, and communities with viable community organizations that might support technical assistance in tilapia culture if were made available.

Analysis: We will collect and synthesize detailed field notes using Atlas-ti software. Of particular interest are the verbatim expressions of the participants about fish culture and the ways it might be realized 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 will be interviewed to anticipate and verify the information provided. One of the major constrains is that producers do not keep any records on their production. Participant interviews will be guided by a general set of questions used to cover the same topics with all participants. The interview guide covers general questions about the prospects for fish culture in a locale, particularly in the context of previous experiences or knowledge.

Our report will provide the NGO community with a template for adding tilapia as an enterprise in the repertoire of activities it supports among indigenous peoples. It will suggest ways that universities and public agencies can conduct research and provide training that will reach indigenous communities where tilapia is an appropriate tool for improving livelihoods and food security.

Schedule

The first six months of the project will be devoted to desk study and review of the literature pertaining to fish culture among indigenous peoples in Guatemala. We will be in contact with our colleagues at San Carlos University, Peace Corps Volunteers, and NGOs in Guatemala with programs or interest tilapia culture. Telephone interviews with knowledgeable persons working in indigenous areas of Guatemala will guide our selection of target departments and communities.

The middle six months will focus on data collection. We plan to conduct field work in six indigenous communities in Summer 2005. Data collection and analysis will be complemented by follow-up phone interviews to clarify interpretations and understandings.

The last six months of the project will focus on summarizing the insights and perspectives gained from fieldwork and collaboration with Guatemalan aquaculture professionals, NGO staff, and university researchers. We plan to issue a report on the practice of tilapia culture among indigenous people in the Guatemala highlands.

A Honduran or Guatemalan student will conduct the fieldwork and complete a MS thesis.

We also plan to issue a Spanish-language leaflet to convey the main insights of the study.

Regional and Global Integration

We plan a formal integration of this activity with the overall region. The work will be jointly conducted by Zamorano personnel (faculty, staff and students). In-country personnel (D. Meyer and S. Meyer) will assist in organizing all training sessions and in development of training materials. The training events will contribute to strengthening our contacts in countries where we have previously interacted and provide opportunities for new contacts in countries that have not had prior relations with the CRSP. Following is a list of Guatemala organization that we have contact with and intend to work with. (They were invited to a course offered in February 2003)

We will endeavor to introduce mixed sex, hand-sexed and sex-reversed production techniques where appropriate. If a commercial producer utilizes or seeks to utilize sex-reversal technologies, we will support these aspirations insofar as they are scientifically sound and legal. For indigenous communities seeking to produce their own fingerlings and among better producers seeking to become area suppliers for small-scale producers, we will provide guidance on hand-sexing and mixed-sex techniques

NGOs and Other Institutions	Kind of Assistance
Pastoral de la Tierra, Catholic Church NGO	Community development
CAESA (NGO, supported by Guatemala Government)	Support poor people in Jalapa
Viejo Quetzal NGO supported by the Catholic church	Assist campesinos displaced by internal war
Christian Church of Chiquimula	Support poor people of Ocotán and Camotán
Proyecto El Estor, NGO supported by the Ministry of Agriculture	Broad set of projects
CARE de Guatemala	Broad set of projects
Movimundo	Assist poor in Altiplano and Quiche
Vivamos Mejor	Support fishermen in Atitlán Lake, tilapia culture in Cages

Unipesa, Aquaculture and Fisheries unit of the Ministry of Agriculture of Guatemala	Support with training and some extension
Marine-culture and aquaculture Center (CEMA) of San Carlos University	Support for farmers with fingerling production and distribution

Schedule

Work will commence October 2004.
Initial tasks will be completed by July 2005.
The remaining tasks will be completed by June 2006.
Report Submission: Quarterly progress reports will be submitted and a final report will be submitted at the end of the second year (June 2006). A report, spanning the two years of this activity is planned to be submitted to the Aquaculture CRSP by 30 June 2006.

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Endnotes

[1] 40 per cent of the indigenous people of Guatemala live in extreme poverty and 74 per cent in poverty as defined by the UN; indigenous women are more affected than men. Three quarters of those facing extreme poverty are indigenous (Stavenhagen, 2002). Over the years, the Indigenous peoples of Guatemala have learned to expect very little from their government and judicial system. The justice system is corrupt; proceedings are rarely conducted in indigenous languages; furthermore, proceedings are unnecessarily lengthy and subject to obstruction and manipulation, and indigenous persons rarely have the resources to employ lawyers, to file various legal papers, or to make court appearances. In addition, those who seek justice are often victimized themselves. As a result, a climate of impunity continues to prevail in Guatemala for most human rights violations, including those perpetrated during the civil conflict, which lasted over a period of more than 30 years. (Amnesty International, 2004).

[2] Guatemala's wet season runs roughly from May – November; the dry season from December – April.

First Annual Sustainable Aquaculture Technology Transfer Workshop

Sustainable Development and Food Security 4 (11.5SDF4)/Activity/Mexico

Investigators

Eunice Perez-Sanchez	HC Principal Investigator	Universidad Juarez Autonoma de Tabasco
Margarita Cervantes Trujano	HC Principal Co-Investigator	Instituto Tecnologico del Mar, Veracruz
Dale Baker	US Principal Investigator	NY SeaGrant
Michael B. Timmons	US Co-Principal Investigator	Cornell University
Kevin Fitzsimmons	US Co-Principal Investigator	University of Arizona
Barry Costa-Pierce	US Co-Principal Investigator	URI SeaGrant
Martin Schreibman	US Co-Principal Investigator	Brooklyn College
David Belcher	US Co-Principal Investigator	Cornell University
Martin Hevia	HC Co-Principal Investigator	La Fundacion Chile

Objectives

1) Prepare materials and lectures for aquaculture technology transfer workshop to be hosted by the Center for Aquaculture Technology Transfer (identify potential research and extension attendees, identify sustainable aquaculture research and extension subjects and presenters, identify location and conference activities, determine synergistic organization meeting potential opportunities).
2) Conduct the 1st Annual Sustainable Aquaculture Technology Transfer Workshop based on Cornell University's annual Recirculating Aquaculture Short Course.
3) Evaluate effectiveness of workshop (interviews, correspondence, update CATT website).

Significance

Large market pressure to supply additional seafood beyond the oceans' limited fisheries to meet the world's increasing population demand for healthy nutritious food can lead to hasty and inadequate aquaculture project planning that has limited concern and negative consequences for the environment. The FAO predicts seafood demand to be approximately 150 million tons by 2010 compared to the current ocean supply of approximately 90 million tons and current aquaculture supply of approximately 40 million tons (FAO source). Inadequate aquaculture project planning can result in environmental degradation, disease outbreaks, and economic failure, such as those experienced in the North/Central/South American and Asian shrimp aquaculture industries during the past 15 years.

One of the most recent trends in aquaculture production development is the use of recirculating aquaculture systems (RAS). RAS based aquaculture production is a sustainable form of aquaculture whereby the aquatic environment is all or partially controlled with little or no water exchange with the outside environment. RAS facilities have integrated water treatment devices to remove solids and pollutants and methods for adding oxygen before the water is returned to the production tanks (Timmons, 2002). A key aspect of RAS is the ability to control pollutant discharge loading. RAS facilities can thus offer unparalleled control over every aspect of water quality from temperature to oxygen concentration, which can be important aspects for optimized aquatic species growth under disease-free conditions.

There are many opportunities for RAS system production in Mexico. Although considered high tech and capital intensive compared to extensive pond production techniques, RAS technology is viewed as a must for commercial aquaculture development where strict environmental control is needed at larval and nursery stage production. This is seen in the international shrimp and salmon production industries where in the wake of diseases such as white spot virus, many farms now demand hatchery-reared rather than pond or wild post larvae or smolt to reduce the risk of disease outbreak in ponds or net pens. (Courtland, 1999) In addition to the commercial production, RAS aquaculture is also applicable for sustainable aquaculture when more conventional forms of aquaculture are not possible such as in areas with a lack of land resources, poor water (pond) retention, excessive source water contamination or an inadequate water supply for conventional aquaculture.

Increased understanding of the basic premises of recirculating aquaculture system technology will also aid in the application of aquaculture, no matter what system approach is used. Often, there are aspects of RAS such as aeration/gas exchange or biofiltration that are applicable to less intensive aquaculture techniques such as flow through raceways and ponds.

Hosting a Technology Transfer workshop featuring recirculating aquaculture system design will be a highly relevant and informative activity of the Center for Technology Transfer (CATT) in its mission of increased sustainable aquaculture extension. In addition to RAS technology, the Technology Transfer workshop will also cover other subjects relative to sustainable aquaculture development such as updates on current CRSP/USAID projects in Mexico and aquaculture entrepreneurship. Entrepreneurial presentations will emphasize small family owned operations in addition to commercial scale operations.

This project will take advantage of the long history of Cornell University being a leader in the development of RAS technology. The project deliverable is to develop a multi-day workshop that teaches the principles of RAS technology and business entrepreneurship with course content specifically developed for Mexico (*Project Theme: Sustainable Development and Food Security*). The course will be couched in a framework of environmental sustainability and job creation through entrepreneurial ventures.

The host country co-principal investigators involved in this project include Margarita Cervantes Trujano of the Instituto Tecnologico del Mar and Eunice Perez-Sanchez of the Universidad Juarez Autonoma de Tabasco. US principal investigators that will assist in the preparation and analysis of the workshop include Michael Timmons of Cornell University, Kevin Fitzsimmons of the University of Arizona, Barry Costa-Pierce of URI/SeaGrant, and Martin Schreibmann of Brooklyn College. Martin Hevia of La Fundacion Chile will also be a Co-Principal Investigator of this project representing the country of Chile. Dr. Hevia is the director of the Spanish distance learning portion of the Cornell Short Course.

Anticipated Benefits

Conducting a sustainable technology transfer workshop will aid in the sustainable aquaculture extension effort of Mexico. This annual workshop will be patterned after the highly successful Cornell University/Freshwater Institute Recirculating Aquaculture Short Course that has been instrumental in educating over 400 aquaculture educators, researchers and entrepreneurial farmers from around the world on recirculating aquaculture production techniques during the past 10 years. The target audience of this workshop will be the researchers and extension personnel of Mexico that are currently involved or intend to be involved in sustainable aquaculture research and production. This workshop will serve to both a) provide technical knowledge to this audience about RAS techniques and b) link and transfer information from existing aquaculture research being performed throughout Mexico. In addition, this technology transfer workshop will also strengthen the research and extension ties among the various research universities and institutions of Mexico and provide an additional link to US resources.

Activity Plan

Activity 1. Prepare Materials and Lectures for Workshop

The first step of this project will be to identify the goals, subject areas, presenters, and attendees of the technology transfer workshop. Initiation of this task will be begin by the HC cooperators and CATT members at the initial CATT organization meeting. The target audience of the workshop will be current and potential aquaculture researchers and extension agents however it will also be open to the larger aquaculture community on a case by case review, i.e., the major focus in the first workshop is to work with the educators. Based on Cornell Short Course experience, it is expected that the first year the majority of attendees will be extension personnel. As the annual workshop continues, the audience will slowly begin to change towards one of more producer participation. The central theme upon which the workshop will be based is sustainable aquaculture technology for development. The primary feature of the workshop will be RAS technology. Content will be changed over time based upon need, e.g., application of GIS tools to assist in aquaculture development planning.

Based upon current national interest and research that is being performed in Mexico through CRSP/

USAID, a strong element of the technical conference will be tilapia culture. In addition to the technology aspects of aquaculture, sessions and presentations on aquaculture entrepreneurship such as fundraising and business plan development will also be provided at the workshop. Holding an aquaculture technology workshop may also provide the opportunity for synergistic organizational meetings. Depending upon membership attendance, a meeting of the CATT extension network membership may be held during the workshop.

The workshop will be held in a central location such as Veracruz over 4 days during December 2005. Preparation for the workshop will include producing all materials and handouts for the presentations and making local logistics. Tours to local aquaculture research or production facilities will also be arranged during this time. Government and industry sponsorship of the workshop will be sought to keep attendance costs as low as possible to allow maximum attendance of the workshop. Attendance and sponsorship fees will be used to offset production costs and to make this annual event a self-sustaining program.

All host country, Chilean, and U.S. co-principal investigators (Cervantes Trujano, Perez-Sanchez, Hevia, Timmons, Fitzsimmons, Costa-Pierce, Schreibman, and Belcher) will participate in this task of the investigation. Workshop preparation is expected to take place over several months in the fall of 2005 in preparation for the December 2005 workshop.

<u>Activity 2. Conduct the First Annual Sustainable Technology Transfer Workshop</u>

The lead presenter of the multi-day workshop will be Dr. Michael Timmons of Cornell University. Dr. Timmons is an internationally-recognized leader in recirculating aquaculture technology and is the founder/chief organizer of the Cornell University/Freshwater Institute Recirculating Aquaculture Short Course which is held each year in the US or Canada. The Cornell Short Course is a one-week course consisting of hands-on and classroom instruction that is intended to give a thorough coverage of the design, operation and management of water reuse systems for finfish. Topics covered in Cornell Short Course include: overview of recirculation systems engineering; water quality monitoring and measurement; engineering design of individual unit processes; systems; fish health management; and economic and risk evaluation.

A similar version of the Cornell Short Course but with added emphasis on business and entrepreneurship will be tailored for the needs of Mexico CATT workshop. A Spanish distance learning segment of the Cornell Short Course that has already been developed by project collaborator Dr. Martin Hevia of La Fundacion Chile will aid significantly in this effort (see attachment below).

Other project members that will participate in the workshop with an emphasis on sustainable technology transfer will include members of ongoing CRSP/USAID research projects in Mexico such as Dr. Kevin Fitzsimmons of the University of Arizona and Dr. Barry Costa-Pierce of the University of Rhode Island SeaGrant. Topics that will be covered by these speakers include tilapia cage culture and polyculture of tilapia and shrimp (Fitzsimmons) and the role of small-scale aquaculture production (Costa-Pierce). Other technical and production related information will also be presented at this workshop depending upon CATT extension network membership identified recommendations.

In addition to the technical presentations, topics on entrepreneurship as it relates to sustainable aquaculture will also be covered at this workshop. Topics to be covered under this portion of the workshop will include fundraising, marketing, and business planning.

Project co-principal investigators that will take the lead on organizing the conference include the host country participants, Cervantes Trujano and Perez-Sanchez with support of US and Chilean team members Timmons, Jacob, Fitzsimmons, Costa-Pierce, and Hevia.

<u>Activity 3. Evaluate the Aquaculture Technology Transfer Workshop</u>

An important element of this investigation will be the evaluation of the efficacy of the workshop. In particular, the investigators will seek detailed feedback and information from workshop attendees on the effectiveness and value of this type of activity so as to improve the workshop for the following year (to be an annual event). Feedback from attendees will be obtained through written comment forms and personal interviews, both during and following the workshop. Feedback questions will be designed for anonymous and objective input (both weighted ranking answers and written comment) and will be compiled in electronic form (MS Excel). Host country and US Co-Principal Investigator analysis of workshop evaluations will be used to determine the success of this first technical workshop as well as the need, type, and structure of our following year's extension workshop (this should be a self-supporting annual event).

Compilation of the workshop feedback will be performed mostly by the host country PI and co-PIs Cervantes Trujano and Perez-Sanchez. All project members will take part in the analysis of the evaluations. Total anticipated time required to retrieve information, analyze the workshop evaluations and provide future recommendations is three weeks (completion by end of February 2006, depending upon actual date of workshop).

Project Schedule

	2005			2006	
Project Timetable	Q2	Q3	Q4	Q1	Q2
Plan for Aquaculture Tech Transfer Workshop			X	X	
Hold Aquaculture Workshop (Veracruz)				X	
Evaluate Performance of Aquaculture Workshop				X	X
Write Final Report (Including analysis of workshop)					X

Regional Integration

In addition to technical information about recirculating aquaculture systems, the technical workshop will include research and extension information from throughout Mexico. In particular, relevant CRSP work that is currently underway on the West and East coasts of Mexico will be presented. The workshop will be open to all interested parties who seek information helpful for sustainable aquaculture development. It is expected that key extension personnel and aquaculture entrepreneurs that are interested in recirculating aquaculture systems and tilapia production will attend this conference and that this conference will serve to unite and strengthen the extension service of Mexico. Participation of US and Chilean collaborators in this project will also provide a broader aquaculture technology link outside the country of Mexico.

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

Production System Design and Integration 1 (11.5PSD1)/Activity, Experiment/Bangladesh, Thailand, and Vietnam

Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology
Nguyen Thanh Phuong	HC Principal Investigator	Cantho University, Vietnam
Md. Abdul Wahab	HC Principal Investigator	Bangladesh Agricultural University
C.Kwei Lin	US Principal Investigator	University of Michigan
James S. Diana	US Principal Investigator	University of Michigan

Objectives

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

Significance

The freshwater prawn (*Macrobrachium rosenbergii*) is indigenous to most Southeast Asian and the south Pacific countries (New, 1982). Since its successful domestication in late 1960s (Ling, 1969) the culture of freshwater prawn has gained a great popularity world wide, mostly in the tropics and subtropical regions, with limited production in temperate regions such as North America (D'Abramo et al., 1989). In recent years the global production of freshwater prawn has increased steadily (FAO, 1996) with the major production in East and South Asian countries--China, India, Indonesia, Bangladesh, Thailand and Philippines. Consisting primarily of *Macrobrachium*, freshwater crustacean production in the region reached 0.5 million tonnes (FAO, 1998). With FAO's efforts on development freshwater prawn culture, a center for research and training was established in Thailand to serve South East Asia in 1970s and 80s. A number of production systems were developed over the years including primarily pond, pen and paddy culture. By far, the most prevailing production system has been intensive pond culture. The widely practiced standard technologies for pond culture involve stocking with hatchery seed, feeding with formulated diets and maintaining water quality by frequent exchanges (New and Singholka, 1985). On average, annual production may reach 2,000 kg/cycle. New (1991) also provided a review on prawn farming.

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

Wastewater from intensive fish culture has been shown to be effective for producing phytoplankton to support Nile tilapia (*Oreochromis niloticus*) culture (Lin et al., 1990; Lin and Diana, 1995; Yi et al., 2001). Apparently benefiting from the nutrient rich effluents, aquatic marcophytes also grow profusely in the areas where effluents are discharged. Water mimosa (*Neptunia oleracea*) and water chestnut (*Trapa bispinosa*) are widely cultivated in fertilized tanks and ponds, and harvested for human consumption in the region. To mitigate the problems of wastewater discharge that pollutes surrounding areas, we propose a new closed system with circulators whereby effluents from freshwater prawn ponds could be recirculated for culture of tilapia, water mimosa and water chestnut. Such diversification and integration are regarded as important practices to enhance sustainable aquaculture (Alder et al., 1996; Pillay, 1996). We will also survey existing farms to better understand current production systems.

Anticipated Benefits

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

Research Design

Location: AIT, Thailand

Methods: Survey:

- Field survey areas: major prawn farming areas in Bangladesh, Thailand and Vietnam. One hundred farms will be randomly selected from the major prawn farming areas in Bangladesh, Thailand and Vietnam, respectively.
- Data collection: Existing geographical information in each area will be collected. A structured checklist and open-ended type of questionnaires will be used to interview farmers to collect the information on topography and land use, climate conditions, farm operational profile, seed supply, culture systems, source water and water management, pond management, feed and feeding management, waste management, and socio-economic conditions of farmers.
- Data analysis: Descriptive statistical methods.

Impact Indicators: New systems should result in greater prawn production, better water quality, lower concentration of nutrient and organic matter in pond effluents, and better economic return compared to traditional systems.

Regional Integration

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

Schedule

September 2004 – May 2006; report submission: 30 May 2006.

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Optimization of Fertilization Regimes in Fertilized Nile Tilapia Ponds with Supplemental Feed

Production System Design and Integration 2 (11.5PSD2)/Experiment/Thailand

Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology
James S. Diana	US Principal Investigator	University of Michigan
C. Kwei Lin	US Principal Investigator	University of Michigan

Objectives

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

Significance

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

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

Anticipated Benefits

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

Research Design

Location: AIT

Methods:

Experiment B. Optimization of Phosphorus Rate in the Presence of Optimal Nitrogen Rate in Fertilized Nile Tilapia Ponds with Supplemental Feed. Pond Research

Pond Facility: 15 earthen ponds of 200 m^2 in surface area.

Culture Period: 180 days.

Test Species: Nile tilapia (*Oreochromis niloticus*)

Stocking Density: 3 fish/m^2.

Nutrient Input: Ponds will be fertilized using urea and TSP at fixed rate of 28 kg N and 7 kg P$\times ha^{-1} \times wk^{-1}$ in all ponds until tilapia reach 100g. Then tilapia will be fed at feeding rates of 50% ad libitum with floating pelleted feed (30% crude protein) in all ponds, and phosphorus rates will be adjusted in various treatments, while the optimal nitrogen rate determined in Experiment A will be used.

Water Management: Maintain at 1-m depth.

Sampling Schedules: Water quality: standard CRSP protocol, biweekly water quality sampling and monthly diel analysis at various depths. Soil samples will be taken and analyzed for moisture, TN and TP at the beginning and end of the experiment.

Fish Growth: monthly sampling and total harvests. Partial economic budgets will be assessed. Nutrient budget will be analyzed. Nutrients contained in pond effluent will be determined by taking water samples at different times from pump outlets during water discharge at harvest.

Statistical Design, Null Hypothesis, Statistical Analysis: The experiment is a completely randomized design with five phosphorus rates as treatments with three replicates each: 0%, 25%, 50%, 75% and 100% of 7 kg P$\times ha^{-1} \times wk^{-1}$, that is, 0, 1.75, 3.50, 5.25, and 7 kg P$\times ha^{-1} \times wk^{-1}$.

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

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

Impact Indicators

Number of fertilization regimes that result in reduction of fertilizer use but maintain or improve tilapia production in comparison with the fertilization regime at fix rates.

Regional Integration

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

Schedule

September 2004 to May 2006. Report submission: May 2006.

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Use of Rice Straw as a Resource for Freshwater Pond Culture

Production System Design and Integration 3 (11.5PSD3)/Experiment/Bangladesh and Thailand

Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology
Md. Abdul Wahab	HC Principal Investigator	Bangladesh Agricultural University
James S. Diana	US Principal Investigator	University of Michigan
C.Kwei Lin	US Principal Investigator	University of Michiga

Objectives

1) Assess the effects of rice straw on fish production, water quality, plankton, bacterial biofilm and periphyton.
2) Optimize the loading of rice straw in both Nile tilapia monoculture ponds and carp polyculture ponds.
3) Compare rice straw and bamboo sticks as substrates in periphyton-based culture systems.

Significance

Rice has been widely cultivated in many Asian countries. Rice straw as a by-product of rice crop is commonly available and a low-cost material. In practice, straws are often burnt in the field or removed for other household uses. Little research has been done to explore the potential of using rice straw to enhance fish production. Recently, rice straw was used as material to cover pond dikes and prevent clay turbidity caused by runoff (Lin et al., 2000; Yi et al., in press) and as substrate to promote bacterial biofilm in periphyton-based culture ponds (Ramesh et al., 1999).

Clay or mud turbidity is a major problem for fertilization management in freshwater ponds. Colloidal clay particles in the water column curtail fertilizer effects and inhibit plankton growth by reducing light penetration and by binding with mineral nutrients from water as well as with plankton cells (Avnimelech et al., 1981, 1982). High clay turbidity usually causes acidity, low nutrient levels, and limited light penetration for photosynthesis (Boyd, 1990), and thus results in reduced primary production (Diana et al., 1991). With only fertilizer inputs, turbidity often limits production and growth of fish (Buck, 1956; Banarjea and Ghosh, 1963). From these points of view, mitigation of mud turbidity is essential to enhance and allow normal phytoplankton growth in response to fertilization. Yi et al. (in press) compared several techniques to mitigate clay turbidity problems, found that Nile tilapia (*Oreochromis niloticus*) production in ponds with dikes covered by rice straw was significantly higher than those covered by netting materials, and concluded that covering pond dikes with rice straw not only reduced clay turbidity caused by run-off but also enhanced Nile tilapia growth probably through microbial biofilm developed on the rice straw.

Periphyton-based aquaculture systems offer the possibility of increasing both real primary production and food availability (Wahab et al., 1999). In periphyton-based aquaculture systems, several types of substrates have been used for the development of bacterial biofilm and periphyton, including easily biodegradable sugarcane bagasse, (Ramesh et al., 1999; Keshavanath et al., 2001), dried Eichhornea and rice straw (Ramesh et al., 1999), less biodegradable bamboo (Hem and Avit, 1994; Wahab et al., 1999; Keshavanath et al., 2001), and non biodegradable plastic (Shrestha and Knud-Hansen, 1994). Compared with less and non biodegradable substrates, biodegradable plant substrates providing more fiber and surface area may favor better growth of fish through bacterial biofilm and periphyton.

Rice straw is a low cost material, widely available to most fish farmers in Asian countries, and has large potential to be used to enhance fish production. However, little research has been conducted to investigate the physical, chemical and biological changes caused by rice straw in fish ponds, and the loading of rice straw in fish ponds should be standardized. The purposes of this study are 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 clay turbidity, as well as enhanced biofilm and periphyton development.

Anticipated Benefits
Results of the experiment will develop an appropriate strategy for enhancing fish production in ponds using rice straw. It will benefit fish producers in Asia and other countries, when extended on a large scale.

Research Design
Location: AIT and BAU.

Methods:
Experiment A. Physical, Chemical, and Biological Changes of Water in the Process of the Decomposition of Rice Straw at Various Loading Levels
Tank research at AIT

Tank Facility: 21 cement tanks of 5 m^2 size.

Culture Period: 30 days.

Test Species: No fish stocked.

Stocking Density: None.

Nutrient Input: Weekly fertilization using urea and TSP at 28 kg N and 7 kg P/ha/week. Water management: maintain at 1-m depth.

Water Quality: Standard CRSP protocol, weekly water quality sampling and diel analysis at various depths, daily measurements of DO, pH, temperature and Secchi disk visibility. Quantify plankton, periphyton and bacterial. Nutrients in rice straw will be analyzed at the beginning and end of the trial.

Statistical Design, Null Hypothesis, Statistical Analysis:
- The experiment will be conducted in completely randomized design with seven treatments and three replicates per treatment. The treatments are five levels of rice straw loading, that is, 0, 625, 1250, 2500, 5000, 10000, 20,000 kg/ha (dry matter basis).
- The null hypothesis is that loading levels of rice straw have no effect on the physical, chemical and biological parameters in water.
- The data will be analyzed using ANOVA and regression.

Experiment B. Optimize Loading of Rice Straw Mats in Fertilized Nile Tilapia Ponds
Pond research at AIT

Pond Facility: 18 earthen ponds of 200 m^2 size.

Culture Period: 150 days.

Test Species: Nile tilapia *Oreochromis niloticus*.

Stocking Density: 2 fish/m^2, 10 g in size.

Nutrient Input: Weekly fertilization using urea and TSP at 28 kg N and 7 kg P/ha/week.

Water Management: maintain at 1-m depth.

Water Quality: Standard CRSP protocol, biweekly water quality sampling and monthly diel analysis at various depths. Quantify plankton, periphyton and bacterial.

Fish Growth: No sampling, based on initial stock and final harvest. Nutrients in rice straw will be analyzed at the beginning and end of the trial. Partial economic budgets will be assessed.

S*tatistical Design, Null Hypothesis, Statistical Analysis*:

- Rice straw will be used to make mats of 2x1 m (LxW) using bamboo frames.
- The experiment will be conducted in completely randomized design with six treatments and three replicates per treatment. The treatments are: (A) no rice straw mats (control); (B) use rice straw mats to cover dikes only; (C) suspend X number of rice straw mats in water column with the equal distance; (D) suspend 2X number of rice straw mats in water column with the equal distance; (E) suspend 3X number of rice straw mats in water column with the equal distance; (F) suspend 4X number of rice straw mats in water column with the equal distance. The number of rice straw mats in a pond will depend on the results of Experiment A.
- The null hypothesis is that different loading levels of rice straw mats have no effect on fish growth, mitigation of clay turbidity, development of bacterial biofilm and periphyton, water quality and economic return.
- The data will be analyzed using ANOVA and regression.

<u>Experiment C. Optimization of Loading of Rice Straw Mats, and Comparison of Rice Straw Mats and Bamboo Sticks Used as Substrates for Enhancing Fish Growth in Fertilized Carp Polyculture Ponds</u>
Pond research at BAU

Pond Facility: 18 earthen ponds of 100 m^2 size at BAU.

Culture Period: 180 days.

Test Species: Silver carp (*Hypophthalmichthys molitrix*), catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), and common carp (*Cyprinus carpio*); species ratio: 1:2:3:2:2.

Stocking Density: 1 fish/m^2.

Nutrient Input: fortnight application of 1,250 kg cow manure, 31 kg urea and 16 kg TSP per hectare.

Water Management: maintain at 1-m depth.

Sampling Schedules: same as f2.2.7.

Statistical Design, Null Hypothesis, Statistical Analysis: Same as f2.2.8.

<u>Experiment D: Comparison of Rice Straw and Bamboo Sticks Used as Substrates for Enhancing Growth of Carps Through Bacterial Biofilm and Periphyton in Fertilized Carp Polyculture Ponds</u>
Pond research at BAU

Pond Facility: 9 earthen ponds of 100 m^2 size at BAU

f.2.4.2 –f.2.4.7: Same as f.2.3.2 - f.2.3.7.

Statistical Design, Null Hypothesis, Statistical Analysis:

- The best treatment from Experiment C will be used to compare to the developed periphyton-based culture system using bamboo sticks as the substrate by Wahab et al. (1999). This experiment may be conducted simultaneously as Experiment C if enough ponds are available.
- The experiment will be conducted in completely randomized design. There will be three treatments in triplicates each: (A) no substrate (control); (B) the best treatment using rice straw mats as substrate from experiment C; (C) Periphyton-based culture system using bamboo as substrate.
- The null hypothesis is that there are significant differences in the growth of all carp species,

mitigation of clay turbidity, development of bacterial biofilm and periphyton, water quality and economic return among the control and the treatments using different substrates.

- The data will be analyzed using ANOVA.

Regional Integration

In many Asian countries, rice straw is a low-cost material and widely available. This experiment will provide optimal loading of rice straw in fish ponds to allow small-scale farmers to achieve higher economic return compared to current systems. It will be used by AIT outreach and country extension agents to advise farmers of better culture practices.

Schedule

Data collection: September 2004 to May 2006. Final report 30 May 2006.

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Development of a Recirculating Aquaculture System Module for Family/ Multi-Family Use

Production System Design and Integration 4 (11.5PSD4)/Activity/Mexico

Investigators

Margarita Cervantes Trujano	HC Principal Investigator	Instituto Tecnologico del Mar, Veracruz
Eunice Perez-Sanchez	HC Principal Co-Investigator	Universidad Juarez Autonoma de Tabasco
Dale Baker	US Principal Investigator	NY SeaGrant
Michael B. Timmons	US Co-Principal Investigator	Cornell University
David Belcher	US Co-Principal Investigator	Cornell University

Objectives

1) Identify aquaculture goals of Mexican family/multi-family stakeholders.
2) Design a suitable recirculating aquaculture system module to meet these goals (may be done as part of a special seminar at the technology transfer workshop).
3) Develop a manual for technology implementation and management.
4) Identify stakeholder participants for demonstration projects and determine project performance evaluation criteria.

Significance

Creating jobs, providing healthy food for human consumption, and protecting the environment are universally identified as some of our world's most pressing concerns. Aquaculture will continue to supply an ever increasing percentage of the seafood being consumed. This trend affords the opportunity to do this in an environmentally friendly and sustainable manner.

All aquaculture development is constrained by the available of suitable water resources. Recirculating aquaculture systems (RAS) represent a very sustainable approach to aquaculture production. The primary advantage of recirculating aquaculture is the control of the environment (air and water). Environmental control is important to obtain optimal growth conditions of the target species and keep out unwanted diseases that can spread in the open environment.

Even in well established aquaculture industries such as the salmon, catfish, tilapia, and shrimp farming industries, RAS systems are typically employed when strict environmental control is needed to ensure maximum production efficiency (e.g., nursery stages of development).

Recirculating facilities can have no impact on the surrounding environment if complete recirculation is used. Recirculating facilities discharge very small quantities of water compared to other forms of culture systems, total water use is less than 0.2% of a typical raceway for the same amount of production (Timmons 2002). Because only small volumes of water are discharged, it is possible to treat the effluent efficiently for pollutants and ensure that there are no negative environmental impacts from the facility.

Based upon identification of development opportunities within Mexico that show maximum promise for job creation and economic development through the existing CRSP projects such as the Santa Maria Bay Project and the Polyculture of shrimp and tilapia project, a system production module will be designed for commercial implementation (*Project Theme: Production System Design and Integration*). Initial testing of the RAS production module will take place at one of the host country principal investigator institutions (final determination depends upon identified project goals and RAS limitations, existing graduate student profile, and facility availability). Target species will be tilapia. If supplemental funding can be found, several additional (three to six) system modules will be built and evaluated.

Anticipated Benefits

Design and implementation of small-scale RAS systems as part of sustainable food production at the family/multi-family level could have significant beneficial effects in rural areas of Mexico. If proven to be successful, similar systems can be established throughout Mexico in areas that do not traditionally support aquaculture at the family or multi-family level (e.g., areas of small land availability or well-drained soils). The target species of this investigation, tilapia, is already a popular fish species in Mexico and is known to be a beneficial form of protein for human consumption. In addition to its food value, the resources to grow tilapia such as fingerlings and feed are readily available throughout Mexico.

Activity Plan

Activity 1. Identify Aquaculture Goals of the Family/Multi-Family Stakeholder Community

The first step in designing a recirculating aquaculture system is to determine the aquaculture goals of the single family/multi-family stakeholder community. A significant amount of information towards this goal is expected to be obtained from both the First Annual Sustainable Technology Transfer Workshop and the work being performed by CRSP in the Santa Maria Bay Management Project. The initial concept of a small semi-intensive RAS system for family or multi-family groups is to provide an additional source of protein that integrates effectively with current agriculture practices. A commercial intensive RAS system of 1 hectare can produce 100 to 200 times the biomass of tilapia produced in a one-hectare pond. Such intense systems require sophisticated water treatment and monitoring systems however to minimize crop loss. A semi-intensive system typical of this project would d have a maximum carrying density of 30 kg tilapia/m^3 production tank volume. This density of tilapia would require relatively simple design for water treatment and would greatly reduce the risk associated with more intensive systems (density > 100 kg/m^3).

The outcome of this task will be a report summarizing the goals and constraints of RAS–based sustainable tilapia production at the family/multi-family scale in Mexico. This task will include cross-referencing with existing CRSP projects and identification of application locations.

A graduate student supervised by host country co-PI will perform the majority of this first activity. Dr. Timmons (Cornell University) is expected to serve on the graduate student's committee. It is anticipated that a significant degree of constraint/project identification will be made during the 1st Annual Sustainable Technology Transfer Workshop; however total estimated time to complete this task is two months.

Activity 2. Design a Suitable Recirculating Aquaculture System Module to Meet These Goals

Based upon the goals and typical farming situation of the identified family or multi-family stakeholders established in Activity 1 above, a recirculating aquaculture system will be designed. The RAS system to achieve the goals of this investigation will be relatively simple yet robust in design to allow for maximum growth with low risk under likely grow out conditions. The primary components of the RAS system will include several production tanks, a settling tank, biofilter, pumps, aeration, and gas transfer equipment. The target cost for the systems will be established based upon local considerations, i.e., design will be reflective of regional investment needs and likely available capital.

Activity 3. Develop a Construction/Operations Manual for Project Implementation and Management

A critical piece for ongoing project implementation and success is a "how-to" manual that both extension agents and individual producers can use a on-site resource guide. The primary information to be provided in the manual will include information on how to construct the RAS module (equipment, site requirements, tools, sources, costs), how to operate the RAS module (equipment operation, maintenance, troubleshooting), and how to properly grow tilapia in a RAS module (stocking, feeding, cleaning, harvesting).

RAS Module construction and operations manual preparation will be conducted by the HC Co-principal investigator's graduate student with guidance from the HC and US co-principle investigators. Financial support of graduate student tuition will be provided through this project for the 16 month project duration.

Activity 4. Identify Stakeholder Participants for Demonstration Projects and Determine Project Performance Evaluation Criteria

The next step of the investigation will be to identify the locations and stakeholders to operate the demonstration RAS modules. The test module will serve as a demonstration facility for technology transfer to the private sector. The parameters that will be measured as part of RAS module evaluation include capital costs, detailed operating costs, time-dependent water quality, and biomass production results. The goal of initial module evaluation is to test system performance and economics and provide further refinement of RAS design and operating guidelines

Module operation location will be determined by the HC Co-Principal Investigator's graduate student with support of the HC and US-based co-principle investigators. Financial support of graduate student tuition will be provided through this project for the 16 month project duration.

Project Schedule

	2005			2006	
Project Timetable (months)	Q2	Q3	Q4	Q1	Q2
Identify RAS goals for stakeholders		X	X	X	
Design and cost RAS module			X	X	X
Develop construction and operations manual			X	X	X
Identify stakeholder participants for demonstration project Determine project performance evaluation criteria				X	X
Write Final Report					X

Regional Integration

Although the design, construction and testing of the RAS production module will be performed by an aquaculture researcher, the goal of this project is to create a system for field implementation. The design, operations manual, and results of this project will be made available to the public and depending upon supplementary fund availability, additional RAS systems patterned after this test system will be built and operated in multiple application areas. Operation and production information will be made available through the CATT website and information exchange listserv, and at future extension conferences.

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Insulin-Like Growth Factor-1 Gene Expression as a Growth Indicator in Nile Tilapia

Production System Design and Integration 5 (11.5PSD5)/Experiment/Philippines

Investigators

Christopher L. Brown	US Principal Investigator	Florida International University
Remedios Bolivar	HC Principal Investigator	Central Luzon State University
Russell J. Borski	US Principal Investigator	North Carolina State University
Emmanuel M. Vera Cruz	Collaborator	Florida International University

Objectives

Insulin-like growth factor-I (IGF-I) is one the most promising candidates to be used as an instantaneous growth indicator in fish. The progress made thus far by the project has been the development of a sensitive TaqMan real time quantitative reverse transcriptase-polymerace chain reaction (qRT-PCR) assay. This technique was validated to quantify tissue levels of IGF-I mRNA in Nile tilapia. The overall goal of this work is to test the efficacy of TaqMan real time qRT-PCR as means of instantaneously quantifying growth of the fish in the laboratory.

The specific objectives are:

1) Correlate hepatic IGF-I mRNA levels to growth rate in *O. niloticus* as modulated by different feeding levels; and
2) Determine the relationship between photoperiod and hepatic IGF-I mRNA levels of the fish.

Significance

Tilapia (*Oreochromis* spp.) has become increasingly important as a rapid, reliable, and affordable source of protein in developing countries. Many regard the fish as the most promising species in the developing world for freshwater aquaculture in the third millennium. It is also gaining prominence as a protein source in the industrialized nations. Imports into the United States, for example, have increased extremely sharply in recent years. With increasing demand for the fish, international research efforts are focused on efficient production to ensure a stable and sustainable supply in the future. Increased and efficient fish production demands fast growing strains, efficient feeds and feeding protocols, and optimization of the culture environment and other parameters known to impact the growth and health of fish. Evaluation of the effect of a particular parameter on growth usually requires numerous and costly production trials. This has been the case in some of the earlier large-scale grow-out trials within the Aquaculture CRSP. Such growth trials are not only expensive, but generally time consuming and labor-intensive. Aside from the considerable expense involved, research progress is also limited by the length of time required to see gross changes in body weight or length for a specific growth trial (for example, see Brown et al., 2000). Normally this involves controlled laboratory or research scale tests followed by large-scale farm trials; both approaches require proper controls and replication for statistical validity. To save time and money, there is a need, therefore, for the development of a means for rapid and direct assessment of growth of fish over short periods of exposure to the parameter being tested.

The establishment of biotechnology-based tools that can be used in the advancement of agriculture in the developing world meets the criteria of one of the cross-cutting themes in current US Agency for International Development work. It is for this reason that this research work has been funded thus far. Biotechnology is believed to have the potential to help feed hungry nations, not so much by the advancement of controversial new genetically modified or transgenic crops, but through the advancement of science that can support and help promote the development of sustainable agriculture technology.

We have seen that radioimmunoassay of the IGF- I hormone provides a good indication of the growth mechanism in tilapias. The examination of IGF- I mRNA gives us a rapid and reliable shortcut to the examination of the growth status of these fish, since it allows a nearly instantaneous examination of their growth status. We realize that the growth mechanism at the gene expression level is poorly characterized in tilapia, and have therefore added a study in which we will investigate the time course of mRNA expression in response to a change in diet, coupled with a time course of the expression of the IGF- I hormone. This should advance our understanding of the timing of IGF- I gene expression and the timing of the subsequent synthesis and release of IGF- I.

Background Information

The growth mechanism in fish is coordinated by a variety of growth factors/hormones acting in endocrine, paracrine and/or autocrine manner. A major step in this hormonal network is the growth hormone (GH) – insulin-like growth factor (IGF) axis (Duan, 1997). The IGF system is composed of ligands (IGF-I and IGF-II), receptors (IGF-IR and IGF-IIR), and IGF binding proteins (IGFBPs). Of the components of the IGF system, IGF-I is the most promising candidate to use as an instantaneous growth indicator in fish. This growth index candidate is a naturally produced molecule of approximately 70 amino acid proteins that is important regulator of growth and differentiation (Werner and LeRoith, 2000). IGF-I mediates the growth promoting actions of GH as and also regulates excessively high production of GH through a negative feedback mechanism (Rotwein et al., 1994; Duan, 1997; Degger et al., 2000; Fruchtman et al., 2000). Natural GH synthesized in the pituitary gland or exogenous GH introduced into the body enters the blood stream and induces the production of circulating IGF-I from the liver where it is predominantly produced (Duan et al., 1994; Reinecke et al., 1997; Duan, 1998; Chen et al., 2000; Kajimura et al., 2001; Pierce et al., 2004). The hepatic-derived IGF-I is released into the blood where it can bind to IGFBPs (Duan, 1997; Hwa et al., 1999, Degger et al., 2000; Kelly et al., 2001). Upon release from these IGFBPs, IGF-I interacts with IGF-IR and it is this mechanism that mediates the majority of IGF-I actions (LeRoith et al., 1995). Insulin-like growth factor-I, presumably acting in autocrine or paracrine manner, is also locally produced in peripheral tissues such as stomach, muscle, cartilage, gills, kidney, pancreas, brain and pituitary gland and the production is induced by GH and other factors such as nutrition (Sakamoto and Hirano, 1993; McRory and Sherwood, 1994; Negatu and Meier, 1995; Duan, 1997).

Both homologous and heterologous IGF-I's have been shown to promote growth in teleosts (McCormick et al., 1992; Negatu and Meier, 1995; Chen et al., 2000; Degger et al., 2000). Several studies indicated a significant and positive correlation between circulating IGF-I and growth rates (Jones and Clemmons, 1995; Beckman et al., 1998; Shimizu et al., 2000; Larsen et al., 2001; Kajimura et al., 2001). The relationship of IGF levels and growth rate is more consistently correlated than that of GH with growth rate. Growth hormone levels can become dissociated with growth rate under some conditions (e.g. malnutrition; prolonged starvation), under which the correlation of IGF-I with growth persists (Duan and Plisetskaya, 1993; Duan, 1997, 1998). For these reasons, the detection of IGF-I is gaining more appeal as a possible indicator of growth rate in fishes (Shimizu et al., 2000; Larsen et al., 2001). In our previous studies, we cloned a portion of *O. niloticus* IGF-I cDNA and developed a sensitive TaqMan real time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay for measures of IGF-I mRNA abundance. We reported the use of hepatic IGF-I mRNA as a positively correlated instantaneous growth indicator in Nile tilapia, with respect to investigations on growth control by temperature and feeding regimen. This qRT-PCR method of quantifying IGF-I mRNA produces more consistent results than the RNA protection assay.

Aside from nutrition and temperature, growth of fish is affected by photoperiod. Photoperiod provides the fish with signal that stimulates an increase in plasma GH levels (McCormick et al., 1995). In gilthead sea bream, long photoperiods had a positive effect on the somatic growth of the fish (Ginés et al., 2004; Kissil et al., 2001). The increased growth under long photoperiods is achieved through increased food conversion efficiency rather than stimulation of feeding (Ginés et al., 2004; Boeuf and Le Bail, 1999). In some species, the positive effect of long photoperiod is attributed to suppressed sexual maturation of the fish, leading to the greater proportion of energy directed towards somatic rather than gonadal development (Rodriguez, et al., 2001). However, under continuous light, growth of fish is reduced (Ginés et al., 2004; Boyer et al., 1994). This could be due to increased physical activity leading to greater metabolism and reduced food conversion efficiency.

Quantified Anticipated Benefits

The primary benefit of this proposed research work will be the further refinement of a tool or method for quantifying growth in tilapia that does not require costly and time-consuming grow-out experiments. The tool, TaqMan real time qRT-PCR assay, will be capable of providing a clear indication of growth stimulation in the fish within a short time of its exposure to the culture condition. The assay will be a valuable tool to several researchers working towards fast growing tilapia and efficient fish production systems. The use of the assay means less time and cost during the development of efficient fish production systems through optimum culture parameters for the existing and novel genetically improved breeds of the fish. Costs of the qRT-PCR method are familiar, since we have been using this assay regularly now for more than a year. The final indirect beneficiaries are the fish farmers in the Philippines and elsewhere, for increased income and efficient fish production and the fish consumers, for stable and sustainable protein supply. This method will be useable in the Philippines, since Mr. E. Vera Cruz, who does these studies in Florida will be returning to Central Luzon State University, and since the necessary materials are all available there.

Research Design

Location: The proposed work will be conducted at Florida International University, and will complete the doctoral studies of Mr. Emmanuel Vera Cruz there. He remains affiliated with Central Luzon State University, where he has served with distinction as a professor. Upon completion of his doctoral program, Vera Cruz will return to CLSU to continue his studies and to apply the exciting new technology of instantaneous assessment of growth in the tilapia-growing regions of the Philippines. Thus it can be correctly asserted that the present location of the proposed research is in Southern Florida and the ultimate goal is the support and enrichment of the aquaculture industry of the Philippines.

Methods:

Study 1. IGF-I and Growth Modulation Using Different Feeding Levels

The study will determine the relationship of IGF-I to growth of tilapia and examine the accuracy and predictive power of IGF-I measurement as a rapid indicator of growth rate. The fish will be fasted in the holding tank for a period of one month prior to the start of the experiment. They will be fed at 1% of BW, given thrice a week for the first 15 days and food-deprived for the last 15 days. Fingerlings will be distributed randomly into nine, 64-liter aquaria at a density of ten fish per aquarium. Each aquarium will be held at 28^{o}C continuously for the duration of the experiment. The aquaria will be divided into three blocks and the following treatments will be assigned randomly in each block: (i) feeding at satiation, (ii) feeding at 75% of satiation level, and (iii) feeding at 50% of satiation level. Fish in each aquarium will be individually weighed at the start of the study. Three, 3, and 4 fish will be collected and individually weighed from each aquarium on day 7, 14, and 30, respectively. Collected fish will be anesthetized in MS-222 and the liver in each fish will be rapidly removed and snap frozen on liquid nitrogen prior to RNA extraction. IGF-I mRNA will be quantified using the TaqMan real time qRT-PCR assay.

Study 2. Effect of Photoperiod on IGF-I mRNA

The study will determine the effect of photoperiod on the level of IGF-I mRNA in the liver of the fish. The fish will be fasted for one month using the procedure as previously described. Fingerlings will be individually weighed and distributed randomly into six, 64-liter aquaria at a density of six fish per aquarium. The aquaria will divided into three blocks and the following treatments will be assigned randomly in each block: (i) 12 h light, 12 h dark, and (ii) 8 h light, 16 h dark. Temperature will not vary among the photoperiod treatments. The fish will be fed once daily at satiation level. The study will be terminated after two weeks and five fish per aquarium will be anesthetized in MS-222 and the liver will be rapidly removed and snap frozen on liquid nitrogen prior to RNA extraction. IGF-I mRNA will be quantified using the TaqMan real time qRT-PCR assay.

Study 3. Time Course of IGF-I mRNA Synthesis

Hepatic IGF-I mRNA levels have been shown to correlate with growth rate strongly as a function of feeding rate or temperature (Vera Cruz, manuscript submitted). The proposed experiment is designed to further our understanding of the kinetics of this component of the growth mechanism in the Nile tilapia. In the above studies (I and 2) hepatic IGF-I will be examined in its relationship with growth rates in immature male tilapia. Temperature will be held constantly at 28^{o}C.

Twenty-five juvenile tilapia of similar size will be individually weighed and isolated at random in 64-liter glass aquaria (1 fish per aquarium) for ten days. These fish will be fasted for one month as in the previous study, in order to reduce or inhibit growth rate. Following this, all fish will be switched to a rich diet, and they will be fed to satiation, daily. Following the transition to full feeding, fish will be sampled throughout a time-course and analyzed for hepatic IGF-I mRNA and blood. At the following times, fish will be collected for this analysis: six hours, 12 hours, 24 hours, 3 days, 5 days. At each of these times, five fish will be anesthetized in MS-222 and the liver will be rapidly removed and snap frozen on liquid nitrogen prior to RNA extraction. IGF-I mRNA will be quantified later using the previously described TaqMan real time qRT-PCR assay. The mean IGF-I mRNA level five fish will be the basal value. In addition, we will examine the validity of the heterologous (*O. mossambicus* anti-IGF-I) radioimmunoassay for use in this species. The two IGF-I molecules are homologous in their circulating forms.

This study will examine the mRNA levels in hepatic tissues and should enable us to examine its relationship with the blood levels of IGF-I by radioimmunoassay.

Schedule

Duration: 18 months (2005–2006).

The three proposed studies eliminate the need for costly and time-consuming grow-out of fish in order to assess growth. The exception is the first study, in which the correlation of the rate of growth and the expression of the IGF-1 gene will be examined, but proposed studies two and three can be completed in far shorter times. The required equipment for these planned studies (–80° F freezer, spectrophometer, refrigerated ultracentrifuge, etc.) have been borrowed in the past, but new equipment is on order now. We anticipate that all of the proposed work can be completed and the results submitted for approximately three scientific publications within the allotted time. Project end date and final report submission will occur by 30 June 2006.

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Development of Nile Tilapia Fillets as an Export Product for the Philippines

Production System Design and Integration 6 (11.5PSD6)/Experiment/Philippines

Investigators

Christopher L. Brown	US Principal Investigator	Florida International University
Remedios Bolivar	HC Principal Investigator	Central Luzon State University
R. Moncarz	Collaborator	Florida International University, Miami

Objectives

To process whole tilapia into fillets, it is vitally important to produce the fish using two stages of culture: one rearing the fingerlings and the other for the grow-out of fish to about 700-800 grams, which is sufficiently large for fillet production. This contrasts with the production of fish to about 450 grams, which is a prevalent size for locally-produced and consumed tilapia. Specific objectives for this project include:

1) Study the cost/benefit relationship of the two-stage production and harvesting schedule.
2) Examine the relationships of stocking density to production efficiency (both fingerlings and grow-out) using the two-stage production schedule.
3) Compare the use of seine nets and cast nets as sampling devices.

Significance

The Philippine tilapia industry would be stronger and more profitable if it were to enter into the business of production of tilapia fillets for export. Tilapia fillets ranging from 100-200 grams are generally in demand in Southeast Asian markets. Addressing this need would help improve the diversity of tilapia operations in the Philippines, which should help decrease production costs and increase profitability (Bimbao et al., 2000). United States tilapia imports rose by more than threefold between 1992 and 1997 (Hatch and Hanson, 2000) and have risen by 50% in the last year (Fitzsimmons, K. Personal communication). There is an increase demand for tilapia fillet in the many parts of the world and very rapid market development for new product forms and by-products (Fitzsimmons, 2002). The market growth for tilapia fillets continues to expand worldwide, and the production and export of fillets would broaden and diversify the economy of the Philippines by adding an export product and cash flow.

In the Philippines, there is a preference for marketable size tilapia that range from 150-250 grams in size, which is too small to be attractive for conversion into fillets, although fish destined for later fillet export could be used to satisfy local market demand. The use of a two-stage grow-out cycle (fingerling production followed by the production of edible fish could satisfy both the needs for production for local markets (smaller fish) and for an export economy (large fish for fillet production).

Quantified Anticipated Benefits

Farmers in Luzon, Island, the Philippines have been the beneficiaries of work from this group in terms of the development and application of innovative technologies, such as feeding strategies and stocking schedules. These methodologies have reduced the effort and cost required to produce tilapia for local consumption, thereby increasing profitability (for further information, see Bolivar et al., 2004; Brown et al., 2004). The proposed Investigation is an outgrowth of these successful studies.

Through the development of tilapia fillets as an export crop, the economy of the Philippines can be strengthened and diversified. At present the Philippine economy is largely a sustenance economy; that is that most of the production is geared toward the meeting of mostly fundamental local needs. By expanding to the production of tilapia fillets for export, local fish production can be maintained while adding an important cash crop, and consequently lead to the generation of funds in support of the national economy. It is expected that a continuous supply of this product will be made available in either fresh or frozen form to established import markets regionally.

Cost benefit analysis will be carried out with the assistance of R. Moncarz, Department of Economics, Florida International University, Miami.

Research Design

Location of Work: Luzon Island, Republic of the Philippines, under the direct supervision of personnel from Central Luzon State University.

Methods:

Study 1. Two-Stage Culture of Nile Tilapia for the Production of Exportable Fillets

Rationale: Bigger size tilapia will require a longer time to culture but the stocking size in the grow-out phase can be regulated to start at around 100 grams. Henceforth the actual culture period and the cost to farmers may be reduced.

This research will be conducted to determine the feasibility of harvesting tilapia that will suit the size requirement for fillet by having two culture stages. This method will depend in part on stocking larger fingerlings but it will be based on conventional feeding methods, using graded amounts of a rice bran and fishmeal based diet that is provided in conventional tilapia culture. A cost-benefit analysis will be done at the end of the study with the assistance of an economist (Dr. R. Moncarz, FIU, Miami).

Methodology:

Phase I – Rearing of Fingerling of Nile Tilapia in Ponds at Three Stocking Densities

This experiment will use twelve (12) 500 m^{-2} earthen ponds at the FAC, CLSU. Three treatments (stocking density of fry size # 22) replicated 4 times will be tested:

- Treatment 1 – 3 m^{-2}
- Treatment 2 – 5 m^{-2}
- Treatment 3 – 8 m^{-2}

The ponds will be fertilized with inorganic fertilizer at the rate of 28 kg of N ha^{-1} $week^{-1}$ and 5.6 kg of P ha^{-1} $week^{-1}$. Commercial feeds will be provided after 75 days of rearing until four months of culture. Fish sampling will be done once a month. Water quality parameters will be measured once a week. Survival rate and average weight of the fish will be determined by draining ponds at the end of the experiment. Processing wastes will be sold for use in agricultural feeds.

Phase II - Grow-out Culture of Nile Tilapia in Ponds

This study will use twelve ponds (12) of 500 m^{-2} each. Three treatments (stocking density) with four replicates will be evaluated.

- Treatment 1 – 1 m^{-2}
- Treatment 2 – 2 m^{-2}
- Treatment 3 – 3 m^{-2}

Stocking size will be on the average 100 g (or 100 ± 10 g). Harvested tilapia from Phase I will be pooled to obtain the required number of fish for stocking. Feeding will be done daily using FeedMix feed at the rate of 5% of the average body weight per day. The ponds will be fertilized with inorganic fertilizer at the rate of 28 kg of N ha^{-1} $week^{-1}$ and 5.6 kg of P ha^{-1} $week^{-1}$.

Water quality parameters will be measured once a week. These will include Secchi disk measurements, determinations of dissolved oxygen, temperature, pH, total ammonia nitrogen, alkalinity, and phosphate determinations. Survival rate and average weight of the fish will be determined. This test will show our ability to produce larger tilapia and hence fillets, but test marketing for export of the fillets is beyond the scope of the present study. Some will be marketed locally, though.

Study 2. Comparison of the Use of Cast-Net and Seine Net as Sampling Devices

Rationale: The seine net has been the traditional device that is used to sample fish from the ponds. The use of cast net for this purpose is becoming increasingly popular for reasons that include reduced rates of stress and injury on the fish and less disturbance on the pond ecosystem. This study aims to compare two sampling devices (cast net and seine net) according to catchability and injury on the fish. Comparisons will be made at approximately the same time of day for consistency.

Methodology: Six 500 m^2 ponds will be used in this study. Tilapia fry will be stocked at size #22 at the rate of 4 fish m^{-2}. Sampling will be done once a month. For each sampling, 5% of the fish population will be sampled. At the end of four months culture period, all fish will be counted and bulk-weighed. 5% will be individually weighed.

This study will use different sizes of cast net accordingly. The mesh size of the seine net will be the same throughout the study.

Study 3. Tank Nursery of Nile Tilapia

This study aims to determine the stocking density and survival rate of tilapia fry in the tank at a stocking size of 22. Twelve concrete tanks measuring 1 x 2 x 2 m will be used in this study. The tanks will be aerated. This study will also be done in the 1 m^{-2} concrete tanks following the same treatment. Four stocking densities (treatment) will be evaluated. Each treatment will be replicated 3 times.

- Treatment 1 – 40 m^{-2}
- Treatment 2 – 80 m^{-2}
- Treatment 3 – 120 m^{-2}
- Treatment 4 – 160 m^{-2}

The tanks will be cleaned and water replenished once daily. Feeding will be done twice daily at percentages of body weight that vary with age.. Water quality parameters will be measured. In the case of increase in pH level, the water will be buffered using hydrated lime.

Survival rate and average size will be determined after three months of rearing in the tanks. This study will start in December 2005 until February 2006.

Study 4. Pond Nursery of Nile Tilapia

This experiment will use twelve 500 m^{-2} earthen ponds at the Freshwater Aquaculture Center, CLSU. Three treatments (stocking density of fry size # 22) replicated 4 times will be tested to grow the fry to size 14. The following stocking densities will be evaluated:

- Treatment 1 – 50 m^{-2}
- Treatment 2 – 75 m^{-2}
- Treatment 3 – 100 m^{-2}

The ponds will be fertilized with inorganic fertilizer at the rate of 28 kg of N ha^{-1} $week^{-1}$ and 5.6 kg of P ha^{-1} $week^{-1}$. No feeding will be done. Fish sampling will be done every two weeks using the appropriate sampling device that has been tested in Study 2. Water quality parameters will be measured once a week. Survival rate, average weight of the fish, the length of culture period to grow the fry to size #14, and the relative production costs will be determined.

Schedule

Duration: 18 months (2005–2006).

The four proposed studies will be run essentially in the order proposed, beginning with study 1. Studies 2 and 4 can be run concurrently over approximately a four month period. Study 3 is a nursery study that can be done in a time-overlapping fashion with the other proposed studies, or after they have been completed. We anticipate that all of the proposed work can be completed within the allotted time. Project end date and final report submission will occur by 30 June 2006.

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Tilapia–Shrimp Polyculture in Negros Occidental, Philippines

Production System Design and Integration 7 (11.5PSD7)/Experiment/Philippines

Investigators

Kevin Fitzsimmons	US Principal Investigator	University of Arizona
Remedios Bolivar	HC Principal Investigator	Central Luzon State University

Objectives

1) Send a graduate student from CLSU to tilapia-shrimp farm on Negros Island to collect data and to corroborate results reported by the industry.
2) Water quality data will be collected to determine if culture of tilapia in conjunction with penaeid shrimp increases the number green algae cells per ml of culture water. We will also attempt to determine if the concentrations of yellow and green fluorescing bacteria are significantly different between treatments.
3) Work with the FYD farm staff to prepare a manual on tilapia-shrimp polyculture based on their system.
4) Share the experimental plan with colleagues in Mexico so that experiments can be coordinated between Philippines and Mexico.
5) Develop an enterprise budget that will compare the costs and benefits of tilapia-shrimp polyculture compared to monoculture of shrimp.

Significance

Tilapia-shrimp polyculture has rapidly spread to most of the tropical shrimp farming countries in response to environmental and disease problems. There appear to be several benefits to stocking tilapia in conjunction with lower densities of shrimp. By contributing to a more sustainable aquaculture system, rearing tilapia with penaeid shrimp would benefit the entire industry (Domingo, 2002). More specifically, returning abandoned ponds to a productive system would benefit local populations who have lost employment with the shrimp farms. It would also ameliorate the loss of natural resources that provided nursery areas for fisheries harvest. The use of green water as produced by the tilapia in the shrimp pond suggest that water quality could be improved and shrimp mortality could be reduced (Bolivar et al., 2003). This project will contribute to the larger goal of developing Sustainable Coastal Aquaculture. Development of mangrove ecosystems for aquaculture and other uses could be slowed or even reversed if we can develop farming methods that utilize polyculture and integrated plant-fish systems to recycle nutrients rather than contributing to eutrophication of aquatic ecosystems. Incorporating seaweeds and mangroves as biofilters and assimilators of nutrients provides for a more comprehensive, ecological approach to the aquaculture system. The efforts within this proposal fit the Investigation description for CRSP purposes.

Quantified Anticipated Benefits

Target Groups:

- Host country aquaculture producers and scientists.
- Our target group are the Filipino farmers formerly employed in commercial shrimp aquaculture.
- Partial financial support for a graduate student to conduct experiments, collect data and prepare a publication.

Quantifiable Direct Benefits from the Experiment:

- Data on stocking rates, growth and survival rates, feed conversion ratios and water quality.
- Provide a manual on tilapia-shrimp polyculture.
- Present and publish results from the trials.

Quantifiable Indirect Benefits from the Experiment:

- Develop sustainable shrimp farming industry for Filipino farmers.
- Demonstrate a model that could be adopted in other countries.

Research Design or Activity Plan

Location of Work: CLSU and FYD Farm near Bacolod, Negros, Philippines.

Methods: This investigation is designed to have a graduate student from CLSU to work with the farm staff at FYD's farm in Negros, where they utilize tilapia-shrimp polyculture as their primary production mode. We will compare their operations and results with similar trials in Mexico and Thailand by MacDonald *et al*. (2004) and Yi *et al*. (2004), respectively. Additional studies of note include those by Domingo (2002) in the Philippines and Thien (2004) in Thailand.With FYD's permission, we will replicate trials in Mexico although there would be several differences between the FYD trial and the trial in Mexico as follows: FYD will use their own hybrid tilapia (*O. mossambicus x O. hornorum*) known as the "Jewel Tilapia" and they will stock *P. monodon* shrimp in a polyculture system. Also, their experimental ponds will be larger while the trial in Mexico will utilize saline tolerant red tilapia and *Litopenaeus vannamei.* The experimental ponds to be used will be smaller in size.

For the FYD trial, ponds with an area of 1 hectare will be used as experimental units. Four replicate ponds will be used for each treatment and four ponds will be used as a control with no tilapia involved. Control ponds will be stocked with shrimp post-larvae at a density of 30 pcs/m^2, and to supply water from the supply channel. Treatment 1 will be stocked with shrimp post-larvae at 30 pcs/m^2, and water will come from a 0.1 ha pond stocked with tilapia at a density of 0.5 pc/m^2 or (5,000 pcs/ha). Treatment 2 will be stocked with shrimp post-larvae at a density of 30 pcs/m^2 and tilapia at 0.5 fish/m^2 (5000 pcs/ha), and make-up water from the supply channel. Treatment 3 will be stocked with shrimp post-larvae at 30 pcs/m^2 and with 1000 "Jewel tilapia" to be stocked each in five (5) hapa cages measuring 10.3 m^3 per cage.

Stocking will attempt to utilize PL 17-18 that are Specific Pathogen-Free *Penaeus monodon* and 5.0 g "Jewel tilapia" fingerlings. Shrimp will be stocked at least three days prior to tilapia being stocked into their respective ponds. This will allow the shrimp to acclimate before the tilapia introductions.

Control ponds will be fed at 10% of the shrimp biomass per day with a weekly adjustment based on sample data. Treatment 1 ponds will also be fed at 10% of shrimp biomass, with weekly adjustment based on sample weights. The tilapia in the supply pond will also be fed 10% of their biomass daily with weekly adjustments. Treatment 2 ponds will be fed the total of 10% of the shrimp biomass plus 10% of the tilapia biomass, adjusted weekly. Treatment 3 ponds will be 10% of the shrimp biomass daily while the fish in the cages will be fed 10% of the tilapia biomass daily. Both amounts will be adjusted weekly. All shrimp ponds will receive an equal volume of make-up water to account for evaporation and flushing. The target value for make-up water will be 2% per week, but this will be adjusted as needed for conditions. Obviously the tilapia pond used to supply the four shrimp ponds in Treatment 1 will need 4 times the volume of water as the other ponds. Tilapia and shrimp will be fed with a commercially available shrimp feed (San Miguel, Jewel Feeds or equivalent 40% protein production diet).

Sampling will occur weekly with at least 20 individual shrimp and 20 tilapia collected from each replicate. Animals will be weighed to the nearest 0.1 g and the new biomass estimate will be used in the adjustment of the amount of feeds.

As it will not be possible to harvest all ponds on the same day, one pond from each treatment will be harvested on the same day. Instantaneous growth rates will be calculated along with the total growth.

Physical, chemical and biological water quality parameters will be monitored. Water quality parameters will include turbidity, temperature, pH, salinity, ammonia-nitrogen, nitrite-nitrogen, orthophosphate, alkalinity, hardness, dissolved oxygen, phytoplankton count as well as bacterial profile (i.e. Total Bacterial Count, Total Vibrio Count, Luminous Bacterial Count). Temperature, DO and salinity will be determined twice daily (07:00 and 16:00). Other water quality parameters will be determined on a weekly or bi-weekly basis. Phytoplankton and bacteria counts will be determined monthly.

Statistical Analyses: Growth rates, survival, and final biomass per pond will be compared between treatments for significant differences using Single-way ANOVA. Difference amongst all treatments will be compared with SNK.

A simple enterprise budget will be prepared in order to report the economic impacts of the polyculture system. This will incorporate the costs and benefits derived from the polyculture systems compared to monoculture.

Regional and Global Integration

Results of investigation will be presented at the ISTA 7 in September 2006.
Results will be compared with similar studies conducted in Mexico and Thailand.
Global development of tilapia-shrimp polyculture will correlated with results of these studies.

Schedule

May 2005 – First visit to Negros.
Late-May 2005 - Stock ponds with tilapia and shrimp.
June - October 2005 – Feeding and growth data collection.
Late October 2005 – Harvest first pond from each treatment.
Early November 2005 – Harvest last pond from each treatment.
January 2006 – Stock second trial.
Early May 2006 – Harvest first pond from each treatment.
Mid May 2006 – Harvest tilapia and complete report.
Late May 2006 – Draft of final report.
Mid June 2006 – Submit Final Report.

Literature Cited

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Testing Three Styles of Tilapia–Shrimp Polyculture in Tabasco, Mexico

Production System Design and Integration 8 (11.5PSD8)/Esperiment/Mexico

Investigators

Kevin Fitzsimmons	US Principal Investigator	University of Arizona
Wilfrido Contreras Sanchez	HC Principal Investigator	Universidad Juarez Autonoma Tabasco

Objectives

1) Conduct tilapia-shrimp polyculture experiment in abandoned shrimp ponds in Tabasco. Trials will compare three polyculture systems; sequential, with tilapia in supply pond, simultaneous with tilapia in cages in ponds and simultaneous with tilapia loose in ponds with shrimp.
2) Water quality data will be collected to determine if culture of tilapia in conjunction with penaeid shrimp increases the number green algae cell per ml of culture water. We will also attempt to determine if the concentrations of yellow and green fluorescing bacteria are significantly different between treatments.
3) Repeat the procedures (as closely as possible) being used at the cooperating commercial farm in the Philippines.
4) Develop a simple enterprise budget to report the economic impacts of the polyculture system in comparison to the monoculture trial.

Significance

Tilapia-shrimp polyculture has rapidly spread to most of the tropical shrimp farming countries in response to environmental and disease problems. There appear to be several benefits to stocking tilapia in conjunction with lower densities of shrimp. By contributing to a more sustainable aquaculture system, rearing tilapia with penaeid shrimp would benefit the entire industry. More specifically, returning abandoned ponds to a productive system would benefit local populations who have lost employment with the shrimp farms. It would also ameliorate the loss of natural resources that provided nursery areas for fisheries harvest. This project will contribute to the larger goal of developing Sustainable Coastal Aquaculture. Development of mangrove ecosystems for aquaculture and other uses could be slowed or even reversed if we can develop farming methods that utilize polyculture and integrated plant-fish systems to recycle nutrients rather than contributing to eutrophication of aquatic ecosystems. Incorporating seaweeds and mangroves as biofilters and assimilators of nutrients provides for a more comprehensive, ecological approach to the aquaculture system. The efforts within this proposal fit the investigation description for CRSP purposes.

Quantified Anticipated Benefits

Target Groups:

- Host country aquaculture producers and scientists.
- Our target group are the Mexican employees formerly employed in commercial shrimp aquaculture.
- Partial financial support for a graduate student to assist with experiment.

Quantifiable Direct Benefits from the Experiment:

- Data on stocking rates, growth and survival rates, feed conversion ratios and water quality.
- Provide results for several models for tilapia-shrimp polyculture.
- Present and publish results from the trials.

Quantifiable Indirect Benefits from the Experiment:

- Develop sustainable shrimp farming industry for Mexican farmers.
- Demonstrate a model that could be adopted in other countries.

Research Design or Activity Plan

Location of Work: UJAT and coastal shrimp ponds in Tabasco.

Methods: This investigation is designed to build upon the earlier work conducted by A. MacDonald et al (2004) in Mexico, by Domingo (2002) in the Philippines, and by Yang Yi et al (2002, 2004) and Thien (2004) in Thailand. Dr. Contreras has access to several abandoned ponds formerly used in shrimp culture, along the coast of the Gulf of Mexico. These ponds could be quickly modified for the polyculture trials. Dr. Contreras has matching funds available to pay for the needed modifications. One-tenth hectare ponds will be used as the experimental units. Four replicate ponds will be used for each treatment and four ponds will be used as a control with no tilapia involved.

1) Control ponds stocked with shrimp at 30 PL's/m2, make-up water from supply channel.
2) Treatment 1 will be stocked at 30 PL's/m2, make-up water from a 0.1 ha pond stocked with 0.5 tilapia /m2 (500 fish).
3) Treatment 2 will be stocked at 30 PL's/m2 and tilapia at 0.5 fish/m2 (500 fish), make-up water from supply channel.
4) Treatment 3 will be stocked at 30 PL's/m2 and contain five floating cages (1.0 m3) each containing 100 tilapia.

Stocking will attempt to utilize PL +15 day shrimp (Specific Pathogen Free *Litopenaeus vannamei*) and 5.0 g tilapia fingerlings saline tolerant red variety). Shrimp will be stocked at least three days prior to tilapia being stocked into their respective ponds. This will allow the shrimp to acclimate and before the tilapia introductions.

Control ponds will be fed at 10% of the shrimp biomass per day with a weekly adjustment based on sample data. Treatment 1 ponds will also be fed at 10% of shrimp biomass, with weekly adjustment based on sample weights. The tilapia in the supply pond will also be fed 10% of their biomass daily with weekly adjustments. Treatment 2 ponds will be fed the total of 10% of the shrimp biomass plus 10% of the tilapia biomass, adjusted weekly. Treatment 3 ponds will be 10% of the shrimp biomass daily while the cages will be fed 10% of the tilapia biomass daily. Both amounts will be adjusted weekly. All shrimp ponds will receive an equal volume of make-up water to account for evaporation and flushing. The target value for make-up water will be 2% per week, but this will be adjusted as needed for conditions. Obviously the tilapia pond used to supply the four shrimp ponds in Treatment 1 will need 4 times the volume of water as the other ponds. Tilapia and shrimp will be fed with a commercially available shrimp feed (Silver Cup or equivalent 40% protein production diet). If the co-operators in the Philippines decide to feed their tilapia a separate tilapia diet, we will switch in Mexico as well.

Sampling will occur weekly with at least 20 individual shrimp and 20 tilapia collected for each replicate. Animals will be weighed to the nearest 0.1 g and a new biomass estimate developed.

As it will not be possible to harvest all ponds on the same day, one pond from each treatment will be harvested on the same day. Instantaneous growth rates will be calculated along with the total growth.

Physical, chemical and biological water quality parameters will be monitored. Water quality parameters will include turbidity, temperature, pH, salinity, ammonia-nitrogen, nitrite-nitrogen, orthophosphate, alkalinity, hardness, dissolved oxygen, phytoplankton count as well as bacterial profile (i.e. Total Bacterial Count, Total Vibrio Count, Luminous Bacterial Count). Temperature, DO and salinity will be determined twice daily (07:00 and 16:00). Other water quality parameters will be determined on a weekly or bi-weekly basis. Phytoplankton and bacteria counts will be determined monthly.

Statistical Analyses: Growth rates, survival, and final biomass per pond will be compared between treatments for significant differences using Single-way ANOVA's. Difference amongst all treatments will be compared with SNK.

A simple enterprise budget will be prepared in order to report the economic impacts of the polyculture system. This will incorporate the costs and benefits derived from the polyculture systems compared to monoculture.

Regional and Global Integration

Results of investigation will be presented at the ISTA 7 in September 2006.
Results will be compared with similar studies conducted in Philippines and Thailand.
Global development of tilapia-shrimp polyculture will correlated with results of these studies.

Schedule

May 2005 – Begin ponds modifications.
Late-May 2005 - Stock ponds with tilapia and shrimp.
June - October 2005 – Feeding and growth data collection.
Late October 2005 – Harvest first pond from each treatment.
Early November 2005 – Harvest last pond from each treatment.
January 2006 – Stock second trial.
Early May 2006 – Harvest first pond from each treatment.
Mid May 2006 – Harvest tilapia and complete report.
Late May 2006 – Draft of final report.
Mid June 2006 – Submit Final Report.

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

Indigenous Species Development 1 (11.5ISD1)/Experiment/Thailand and Vietnam

Investigators

Amrit N. Bart	HC Principal Investigator	Asian Institute of Technology
Dinh Van Trung	HC Principal Investigator	Research Institute for Aquaculture No.1 – Vietnam
James S. Diana	US Principal Investigator	University of Michigan

Objectives

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

Significance

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

Southeast Asia is known to possess one of the largest diversity of freshwater fish species in the world (FAO, 1992). Unfortunately, comparatively few species from this region have been brought under cultivation partly due to lack of sufficient knowledge on reproduction and seed production. *Spinibarbus denticulatus*, is an example of such a species which has significant potential for aquaculture, particularly for a low-input system of North Vietnam (Red River Delta System). This herbivorous species has a diet consisting of plankton and macrophytes, very similar to that of the grass carp (Bao, 1978). One of the most attractive features of this species is that it is resistant to red spot disease, even when grown together in the same cage with infected grass carp. It is also a fast growing species and was cultured primarily by stocking in ponds and cages with seed collected from the wild. Culture of this species is constrained by a limited supply of seed in the wild. We know of no publication that characterizes reproduction of this species beyond some basic biology. Preliminary studies at the Research Institute for Aquaculture No. 1 in Hanoi indicate that this species could be spawned in captivity and respond to natural and hormonal stimuli for spawning. A more thorough study is needed to better understand the reproductive biology and to produce seed using low cost and relatively simple hatchery techniques.

Anticipated Benefits

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

1) Lower the seed collection pressure in natural populations;
2) Increase the availability of seed in a more predictable manner at a lower cost, with potential for making this fish more widely cultured throughout Southeast Asia;
3) Replace exotic species such as grass carp; and
4) Add one more low-cost freshwater fish to the list of aquaculture species with the potential to directly benefit the rural poor.

Research Design

Location: Research Institute for Aquaculture No. 1 – Vietnam. This is the lead aquaculture institute in

Vietnam with over 12 trained scientists on location. It has functioning wet and dry lab and a number of well-managed ponds on campus. Additionally, it has several satellite campus where majority of this study will be conducted.

Methods: This study is expected to require 2 years for completion. The first year will provide us with the basic knowledge on hormonal profile, sexual maturation, and gonadal development relative to the breeding season. It will also evaluate responses to environmental and exogenous hormones. In the second year we will optimize the inducing agents (environmental and hormonal) and develop larval rearing techniques.

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

Response to environmental and hormonal stimuli *Environmental parameters tested*- 1) Increase water temperature by lowering of the water depth and 2) Simulate rainfall by increasing the water level and placing a sprinkler system on the 200m^2 pond surface.

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

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

Larval Rearing: newly hatched larvae will be placed in clear and green water systems in the hatchery. Both will be fed with live rotifers five times per day until they are ready to feed on fine particles of artificial feed. Growth rate, feeding vigor and survival will be monitored for 4 weeks post hatch.

Experimental Design, Null Hypothesis, and Statistical Methods:
<u>Experiment 1: Sexual Maturation</u>
Null Hypothesis: there is no seasonal effect on sexual maturation of this species. Sampling will be done every 2 weeks and increase sampling frequency as the spawning season peaks using 10 males and 20 females.

<u>Experiment 2: Environmental Control of Maturation and Spawning</u>
Null Hypothesis: Temperature and rain have no impact on advanced maturation and spawning. Twenty pairs will be placed triplicate ponds. A separate 2 ponds containing 20 pairs each will not receive treatment and function as the control.

<u>Experiment 3</u>: Forty females will receive 3 doses (high-medium-low) of each.
hormones (CP and GnRH) (five fish per treatment). Controls will receive saline injection.
Tables and figures will summarize the information from Experiment 1. Mean significant differences will be tested using ANOVA in Experiments 2 and 3.

This is a cyprinid species similar to grass carp in its feeding habits, growth and flesh texture (except it has no intramuscular bones). It would potentially replace grass carp where there have been difficulties with red spot disease. However, in the regions where grass carp is commonly cultured and not affected by disease, *S. denticulatus* would complement as they can be stocked together and fed the same diet. While commercial potential is unexplored, it has significant implication to resource poor farmers and rural communities in the South and Southeast Asia because of low input required for culture of this species.

Schedule

Experiments 3 and 4 – September 2004 to May 2006.
Report submission: 30 May 2006.

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Broodstock Development of Amazonian Fishes

Indigenous Species Development 2 (11.5ISD2)/Experiment/Colombia

Investigators

Christopher C. Kohler	Project Director and Principal Investigator	Southern Illinois University at Carbondale
William Camargo N.	Project Coordinator and Co-Principal Investigator	Southern Illinois University at Carbondale
Santiago Dúque E.	HC Principal Investigator	Universidad Nacional (Instituto de Investigaciones IMANI), Leticia, Colombia
Juan Carlos Alonso	HC Co-Principal Investigator	Instituto Amazónico de Investigaciones Científicas SINCHI, Leticia, Colombia
Edwin Agudelo Cordoba	HC Co-Principal Investigator	Instituto Amazónico de Investigaciones Científicas SINCHI, Leticia, Colombia
Carlos Augusto Pinto	HC Co-Principal Investigator	Acuarios Leticia, Leticia, Colombia

Objective

1) Improve the quality of progenies by manipulating water quality for gamitana (*Colossoma macropomum*) and/or paco (*Piaractus brachypomus*).

Significance

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 brachypomus (commonly called red 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 attain a high price at the marketplace.

Considering that fish is a major part of the diet of Amazon communities (Brazil and Peru; Eckmann, 1983), studies on aquaculture of fishes will maintain their consumption without overfishing 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 brachypomus (frugivorous fish) are important because the commercial fisheries over-exploits these frugivorous fishes, thus, the population is severely affected as the large size of Colossoma macropomum and Piaractus brachypomus is sold at increasingly higher cost. There is increasing pressure in the conversion of the floodplains to rice paddies and cattle pastures (deforestation; Achard et al., 2002) and viable aquaculture may prevent this trend. Our proposed study could boost the interests in promoting the aquaculture of these indigenous species by providing information on its reproductive requirements.

Quantified Anticipated Benefits

The proposed study aims to investigate key aspects of nutrition and reproduction biology of Colossoma sp., and 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 will be the fish producers in the Amazon region. Development of the technology of intensive growth of these species and stocking 4-6 weeks old juveniles will dramatically increase their survival and efficiency of production.

More importantly, this study will also contribute 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) and Instituto Amazónico de Investigaciones Científicas-SINCHI (Programa de Ecosistemas Acuáticos, Colombia).

Research Design or Activity Plan

Location of Work: Experiments will be conducted in Leticia (Colombia) in the experimental station of a NGO called "Acuarios Leticia" under the supervision of Universidad Nacional de Colombia and SINCHI. Universidad Nacional de Colombia, Acuarios Leticia and SINCHI are non-profit research institutions that are charged to alleviate the socio-economic conditions of the Amazonian farmers by conducting research on sustainable development and protection of natural resources.

Methods:

Objective 1. Improvement of the Quality of Progenies by Manipulating Water Quality for *Colossoma* sp. and/or *Piaractus* sp.

This objective will be carried out in Colombia in the Acuarios Leticia research station in Leticia, under the supervision of Universidad Nacional de Colombia and SINCHI. Water quality will be manipulated to emulate to three different environments (with 3 different alkalinities, pH levels, conductivities, humic acid contents, transparencies and TDS) where these fish inhabit naturally to evaluate water quality as a conditioning factor for fish reproduction. Eighteen Colossoma sp. and/or Piaractus sp. males and females will be pit-tagged and randomly distributed to three ponds. Fish will be induced to spawn by hormonal injections and eggs from individual females incubated separately to monitor percentage of eyed embryos (13 hours after fertilization) and hatching rate. Specific growth rates, food conversion ratios and condition factors of broodstock will be compared between treatment groups. Survival of larvae at the free-swimming stage will be considered as a final indicator of their quality.

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

Regional Integration

Colossoma sp. and *Piaractus* sp. are economically important throughout the Amazon region. Accordingly, studies that promote the artificial propagation of these fishes will improve the profitability of aquaculture operations of fish farmers and consequently economic conditions of rural communities in these countries. In addition, readily available techniques of farming these fishes will reduce the pressure of catching fish from the wild. Results from these studies will be published as fact sheets and distributed to key academic and research institutions in the region to promote interests among researchers in working with these frugivorous fishes.

Schedule

Objective 1. Improvement of the Quality of Progenies by Manipulating Water Quality for *Colossoma* sp. and/or *Piaractus* sp., and/or *Arapaima gigas*

- October–December 2004: Preparation of ponds. Preparation of larval rearing tanks
- January-April and August-November 2005: Observation of spawning behavior of gamitana and/or paco in ponds.
- April–May and September–November 2005: Induction to spawn for gamitana and/or paco. Evaluation of gamitana and/or paco reproductive performance.
- April–May and September–November 2005: Evaluation of gametes/embryo quality.
- June and December 2005- April 2005: Preparation of manuscripts.

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Incorporation of the Native Cichlid *Petenia splendida* into Sustainable Aquaculture: Reproduction Systems, Nutrient Requirements and Feeding Strategies

Indigenous Species Development 3 (11.5ISD3)/Experiment/Mexico

Investigators

Carl B. Schreck	US Principal Investigator	Oregon State University
Guillermo R. Giannico	US Principal Investigator	Oregon State University
Wilfrido M. Contreras Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco
Carlos Alfonso Alvarez González	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco
Gabriel Márquez Couturier	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco
Ulises Hernández Vidal	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco

Objectives

1) Evaluate the reproductive performance of *Petenia splendida* under controlled conditions using several sex ratios.
2) Determine if *Petenia splendida* fry can be successfully cultured using high initial stocking densities.
3) Determine fish meal substitution with vegetable meals for *Petenia splendida* at fry, juvenile and adult stages using practical diets.

Significance

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 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) with 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 a conservation standpoint at a time when the local and foreign demand has imposed great pressures on their natural populations. During the 1990s researchers from the University of Stirling, in collaboration with researchers from the Colegio de Posgraduados, initiated studies in Tabasco to determine if the native cichlid "castarricas" (*Cichlasoma urophthalmus*) could be incorporated into aquacultural practices. After several years of investigation, they recommended reproduction techniques and systems to accomplish this; however, they also suggested that castarricas were not as efficient as tilapia for aquaculture. The incorporation of tenhuayacas (*Petenia splendida*) into aquacultural practices has not been addressed in the past. Despite the interest of local farmers, native cichlids have largely been ignored and mistakenly considered as incapable of competing with tilapia. Because of this, no studies have been performed to determine if this is the case. No economic studies have been conducted despite high demands in the local, regional and national markets. The price for tenhuayaca is double that of tilapia per kilo and it is often requested in markets and restaurants. In addition, fishermen have requested that the State government re-stock lagoons and rivers with this species. At UJAT we have investigated this species for more than four years. However, more research is needed to provide farmers with a technological package to initiate the culture of this species and to provide local governments with high quality fry to recover native tenhuayaca populations.

In Tabasco and Chiapas, several species of native cichlids have been proposed for aquacultural purposes. Among those species, *P. splendida, Cichlasoma synspillum* and *C. urophthalmus* are of special interest because of their local demand and cultural value (Mendoza, 1988). Few studies have evaluated the basic biological parameters for incorporation of new species into aquaculture processes and information is needed in order to participate in the recovery of their populations and launch a different kind of aquaculture; one based on sustainability of native species and food security.

During the last decade the culture of native species has been ignored. Some information has been obtained from wild populations and little is known about the reproductive performance of native species in captivity. In our laboratory, we have studied some reproductive features of *P. splendida, C. synspillum*, and *C. urophthalmus*. We have found indications that tenhuayaca may be a very good candidate for aquaculture purposes. Commercially, tenhuayaca is the most desirable species. Among cichlids, it has the highest value in the local market to the point of competing with snooks in price and the demand is very high.

Preliminary data generated at UJAT, suggests that females produce an average of 3,406 fry per spawn. A masters degree student is currently raising large amounts of fry in 2m-diameter circular tanks and we will determine if the use of plastic covers will allow breeding pairs to build nests. More research is needed to determine which sex ratios to use in the system in order to maximize fry production. The reproductive season for this species starts in July and it has been reported that it ends in October-November (Resendez & Salvadores, 1983). In the wild, both males and females protect their fry for more than 30 days. However, studies at UJAT indicate that if fry are removed from the nest, the female will move away and the male will initiate courtship behavior with other females present in the system. Females are capable of spawning again after two months. Our preliminary data indicate that females can spawn every 28 days.

We have also started studying the acceptance of commercial feeds and growth performance of this species. So far we have compared growth performance of fry fed *Artemia* nauplii against fry fed commercial feed. We have observed a significant difference in growth if the fish are fed nauplii during the first 30 days of feeding, even though fry accepted inert food. Fish fed 30 days with nauplii grew more than twice compared to those that received commercial feed. Empirical information indicates that males grow faster and larger than females, but further research is needed to evaluate the growth of masculinized populations. Tenhuayacas produced at UJAT have been used to stock lagoons, rivers and earthen ponds. The municipal hatchery reported that fry grew up to nine cm in 90 days of grow-out. There is a strong demand for tenhuayaca fry from this hatchery and they have requested 100,000 fry from UJAT. This preliminary information is promising; however, more research is needed to evaluate if tenhuayacas can be as successful in intensive aquaculture systems.

This proposed investigation will address if tenhuayacas can be produced on a large scale. In order to do this three research areas need to be studied: 1) reproductive performance with different sex ratios; 2) intensive fry culture using high stocking densities; and 3) protein requirements for fry, juvenile and adult growth using practical diets. If successful, the technological package developed can be transferred to local hatcheries located in poor areas of the states of Tabasco and Chiapas, México. The use of reliable methods in these hatcheries will have a significant benefit for thousands of small farmers that currently see their productivity limited to the use of tilapia. Small farmers may benefit further because tenhuayacas have a higher price both in local and national fish markets.

The studies included in this investigation aim to increase the sustainability of methods for mass-production of tenhuayaca fry, by establishing grow-out strategies, and by diversifying farm production to take advantage of market opportunities associated with native species.

A series of training workshops will be developed and offered to different audiences in the communities of Tabasco and Chiapas to ensure that the above mentioned methodology is effectively transferred to its final users. Technical workshops will target hatchery managers, extension agents and university students (many of whom will become workshop instructors over time). Public extension workshops will be tailored to the cultural characteristics of the target audience and will be offered to fish farmers, farm workers, and selected community leaders.

Anticipated Benefits

Tenhuayaca fry production will benefit fishermen, farmers and extension agents. For several years tenhuayaca fry have been requested to restore populations in lagoons and rivers. Aquaculturists are also requesting tenhuayacas to include them in aquaculture systems either alone or in polyculture to control tilapia fry production.

Research Design

Study 1. Intensive Spawning and Nursery Techniques for *Petenia splendida*

Experiment 1: Parental Sex Ratios
Location: the Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Three sex ratios will be evaluated to determine the best fry production rates. The male:female ratios to be evaluated will be 1:1, 1:2, and 1:3. Each treatment will consist of three 2.0 m diameter tanks that will be divided into six spawning compartments and a 1 ft^2 plastic mat substrate will be placed as spawning substrate. In the first treatment, 6 females and 6 males will be placed in each replicate; in the second treatment 6 females and 3 males will be placed in each replicate; in the last treatment, 6 females and 2 males will be placed in each replicate. The fish will be allowed to spawn over a six-month period. Each substrate will be observed daily for nesting activity. Substrates found to have eggs will be removed and new substrate added. Dissolved oxygen and temperature will be recorded at 7:00 am and 7:00pm. Brooders will be offered a pelleted diet daily. Males and females will be tagged, weight and total length will be measured and spawning activity will be monitored individually.

Statistical Methods and Hypothesis: The null hypothesis is that there will be no differences in fry production between treatments. Data will be analyzed as to the frequency of female spawning.

Schedule: Data collection, February 2005–June 2006; Final technical report, 30 June 2006.

Experiment 2: *Petenia* Fecundity and Hatching Success
Location: The Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Using spawns obtained in Experiment 1, the fecundity of *P. splendida* will be determined and hatching success established. A 1.5% sodium sulfite solution will be used to remove the adhesive eggs from substrate. A minimum of 10 spawns will be separated from the nest substrate and enumerated. The female producing each spawn will be weighed and fecundity calculated as the number of eggs/g of female. Nests will be transferred to a hatching tray. Fertilized and none fertilized eggs will be counted. The percent hatch and larvae survival to swim-up stage will be determined for spawns incubated.

Statistical Methods and Hypothesis: The null hypothesis is that there will be no difference in egg hatching success or fry survival between females. Percent data will be arcsin transformed and analyzed by ANOVA to determine treatment effects. A linear regression analysis will be conducted to determine the fecundity model.

Schedule: Data collection, February 2005–June 2006; Final technical report, 30 June 2006.

Experiment 3: Effect of Stocking Density on Growth and Survival of *P. splendida* Fry
Location: The Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Nursery tanks will be prepared by filling the tanks with filtered water and adding chicken manure at 500 kg/ha ten days before fish stocking. Additional fertilizer will be added at 250 kg/ha weekly. Sex reversed Petenia larvae will be stocked at densities of 5, 10 and 20/m^2 using 4 replicates/stocking density. Morning dissolved oxygen and temperature will be recorded daily. After 90-d all nursery tanks will be harvested and the total number of fish and their weights determined. A sample of 25 fish /tank will be measured to the nearest mm to determine size variation.

Statistical Methods and Hypothesis: The null hypothesis is that there will be no difference in fry survival, growth, or size variation, as a function of stocking density. Percentage data will be arcsin transformed, data will be analyzed by ANOVA to determine treatment effects.

Schedule: Data collection, April 2005–June 2006; Final technical report, 30 June 2006.

Study 2. Vegetable Meal Utilization of *Petenia splendida* at Different Life Stages

Experiment 1: Effects of Substitution of Fish Meal for Vegetable Meal on Growth and Survival of *Petenia splendida* Fry Fed Practical Diets
Location: Experiment will be conduced at the Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Growth of *P. splendida* fry will be evaluated using practical isocaloric diets containing 25, 50, 75, and 100% wheat meal.

Laboratory and Pond Facility at UJAT: 5 rearing recirculating systems (12, 20-l tanks each); 2 rearing recirculating systems (27, 20-l tanks each).

Culture Period: 30 days or until fry reach 200% of their initial size.

Stocking Rate: 20 fish/l.

Tests Species: *Petenia splendida*

Nutrient Input: None.

Water Management: Water for rearing and growth-out will be recirculated using bio-filters, 25% of the volume will be exchanged twice a week.

Sampling Schedule: Tenhuayaca fry will be sampled at the beginning of the experiment and every 15 days. Total length and weight will be measured to the nearest 0.001 mm or g. Mortality will be recorded daily. Experimental treatments will be as follows:

1) Tenhuayaca fry fed with a diet containing 25% wheat meal and 75% fish meal.
2) Tenhuayaca fry fed with a diet containing 50% wheat meal and 50% fish meal.
3) Tenhuayaca fry fed with a diet containing 75% wheat meal and 25% fish meal.
4) Tenhuayaca fry fed with a diet containing 100% wheat meal.
5) Tenhuayaca fry fed with Artemia nauplii (Control No. 1).
6) Tenhuayaca fry fed with a diet containing 100% fish meal (Control No. 2).

Each treatment will consist of three replicates. First feeding fry will be fed with experimental diets four times a day. Fish will receive rations containing 20 percent of their body weight. Daily rations will be estimated using a spread-sheet constructed with previous growth data.

Statistical Methods and Hypothesis: Ho1: Different vegetable meal levels included in the diets will produce equal growth and survival of *P. splendida* fry. Ha1: Different vegetable meal levels included in the diets will produce different growth and survival of *P. splendida* fry. To test the null hypothesis growth data will be compared using a one-way ANOVA. Survival will be compared using a Chi-square test.

Schedule: Data collection, April 2005–June 2006; Final technical report, 30 June 2006.

Experiment 2: Effects of Substituting Fish Meal for Vegetable Meal on Growth and Survival of *Petenia splendida* Juveniles Fed Practical Diets

Location: Experiment will be conduced at the Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Growth of *P. splendida* juveniles will be evaluated using practical isocaloric diets containing 25, 50, 75, and 100% vegetable meal.

Laboratory and Pond Facility at UJAT: 1 grow-out recirculating system (36, 120-l tanks); 1 grow-out recirculating system (18, 120-l tanks).

Culture Period: 90 days.

Stocking Rate: 1 fish/l.

Tests Species: Tenhuayaca, *Petenia splendida.*

Nutrient Input: None.

Water Management: Water for grow-out will be recirculated using bio-filters, 25% of the volume will be exchanged twice a week.

Sampling Schedule: Tenhuayaca juveniles will be sampled at the beginning of the experiment and every 15 days. Total length and weight will be measured to the nearest 0.001 mm or g. Mortality will be recorded daily. Experimental treatments will be as follows:

1) Tenhuayaca juveniles fed with a diet containing 25% wheat meal and 75% fish meal.
2) Tenhuayaca juveniles fed with a diet containing 50% wheat meal and 50% fish meal.
3) Tenhuayaca juveniles fed with a diet containing 75% wheat meal and 25% fish meal.
4) Tenhuayaca juveniles fed with a diet containing 100% wheat meal.
5) Tenhuayaca juveniles fed with a diet containing 100% fish meal (Control).

Each treatment will consist of three replicates. Juveniles will be fed with experimental diets four times a day. Fish will receive rations containing 10 percent of their body weight. Daily rations will be estimated using a spread-sheet constructed with previous growth data.

Statistical Methods and Hypothesis: Ho1: Different vegetable meal levels included in the diets will produce equal growth and survival of *P. splendida* juveniles. Ha1: Different vegetable meal levels included in the diets will produce different growth and survival of *P. splendida* juveniles. To test the null hypothesis growth data will be compared using a one-way ANOVA. Survival will be compared using a Chi-square test.

Schedule: Data collection, June 2005–June 2006; Final technical report, 30 June 2006.

Experiment 3: Effects of Substitution of Fish Meal for Vegetable Meal on Growth and Survival of *Petenia splendida* Adults Fed Practical Diets

Location: The Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Growth of *P. splendida* adults will be evaluated using practical isocaloric diets containing 20, 30, 40, and 50% protein.

Laboratory and Pond Facility at UJAT: 7 concrete tanks and 1 earth pond.

Culture Period: 90 days.

Stocking Rate: 5 fish/m^3; fish will be stocked in 3x3 m hapas.

Tests Species: Tenhuayaca, *Petenia splendida*

Nutrient Input: None.

Water Management: 25% of the volume will be exchanged twice a week.

Sampling Schedule: Tenhuayaca adults will be sampled at the beginning of the experiment and every 15 days. Total length and weight will be measured to the nearest 0.001 mm or g. Mortality will be recorded daily. Experimental treatments will be as follows:

1) Tenhuayaca adults fed with a diet containing 25% wheat meal and 75% fish meal.
2) Tenhuayaca adults fed with a diet containing 50% wheat meal and 50% fish meal.
3) Tenhuayaca adults fed with a diet containing 75% wheat meal and 25% fish meal.
4) Tenhuayaca adults fed with a diet containing 100% wheat meal.
5) Tenhuayaca adults fed with a diet containing 100% fish meal (Control).

Each treatment will consist of three replicates. Adults will be fed with experimental diets four times a day. Fish will receive rations containing 10 percent of their body weight. Daily rations will be estimated using a spread-sheet constructed with previous growth data.

Statistical Methods and Hypothesis: Ho1: Different vegetable meal levels included in the diets will produce equal growth and survival of *P. splendida* adults. Ha1: Different vegetable levels included in the diets will produce different growth and survival of *P. splendida* adults. To test the null hypothesis growth data will be compared using a one-way ANOVA. Survival will be compared using a Chi-square test. Regression and broken-line analyses for weight data will be used to determine protein dietary requirements of *Petenia* adults.

Schedule: Data collection, June 2005–June 2006; Final technical report, 30 June 2006.

Regional Integration

If the results of the proposed study are successful, we will exchange information with colleagues in central America. we are particularly interested in exchanging information with Daniel Meyer at the Panamerican Agricultural School.

INDIGENOUS SPECIES

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Broodstock Development and Larval Feeding of Amazonian Fishes

Indigenous Species Development 4 (11.5ISD4)/Experiment/Peru

Investigators

Konrad Dabrowski	US Principal Investigator	The Ohio State University
Jacques Rinchard	US Co-Principal Investigator	The Ohio State University
Christopher C. Kohler	Project Director and US Principal Investigator	Southern Illinois University at Carbondale
William Camargo	Project Coordinator	Southern Illinois University at Carbondale
Guillermo Alvarez	HC Principal Investigator	Universidad Nacional Mayor de San Marcos, Lima, Peru
Salvador Tello	HC Principal Investigator	Institute for the Investigation of the Peruvian Amazon
Maria Celia Portella	Collaborator	Sao Paulo State University, Jaboticabal, Brazil

Objectives

1) Determine gametogenesis and gonad differentiation in a captive stock of South American catfish, *Pseudoplatystoma* sp.
2) Determine changes in plasma sex steroid hormones during an annual reproductive cycle in *Pseudoplatystoma* sp.
3) Induce reproduction of *Pseudoplatystoma* sp. and assess blood plasma steroid response and gamete production (egg and sperm quality).
4) Develop larval diets for Amazonian catfishes *Pseudoplatystoma* sp.

Significance

Amazon and Parana Rivers catfishes, *Pseudoplatystoma coruscans*, *P. fasciatum*, and *P. tigrinum*, are potential aquaculture species in South America (Kossowski, 1996; Campos, 2004). These fish produce excellent quality and tasty meat with minimal bones (Martino et al., 2002) making them appreciated in the region. Wild populations have decreased considerably due to overexploitation of stocks along the Amazon (Cerdeira et al., 2000). In Peru, spawning of catfish occurs from February to March and some fish farmers have considerable success in domesticating these fish (Alcantara F., pers. comm.). Carp pituitary extracts and pituitary hormones are used to induce their final maturation and ovulation (Cardoso et al., 1995; Kossowski, 1996; Leonardo et al., 2004). However, to the best of our knowledge, no information is available on the profiles of plasma sex steroids which could potentially be used to synchronize ovulation/spermiation in these fish (Dabrowski et al., 1996). Our preliminary data on plasma sex steroid hormones and gonadal development indicated that the Amazonian catfishes that we currently raise in our aquaculture indoor facility, are undergoing sexual maturation. The annual changes in blood plasma steroids as well as the surge of maturational hormones preceding the spermiation or ovulation can contribute to a better understanding of the dynamics of gonadal steroidogenesis. The determination of sex steroid hormones will be carried out in parallel to histological analysis of gonads and also include biopsies of gonads (catheterization of ovaries). That will provide additional information to already stated description of gametogenesis in South American catfish. Knowledge of steroids and their dynamics would allow better programming "reproduction strategy" in the future when the origin of fish and state of gametogenesis in geographically distant location from their natural habitat are unknown. For instance, there is no evidence, to the best of our knowledge, on the reproduction of *Pseudoplatystoma* on the North American continent. Moreover, such information will be useful in the development and standardization of breeding techniques through the use of natural (carp pituitary) and/or synthetic hormones.

In Brazil, favorable growth rates were observed in *P. coruscans* juveniles in captivity (Fujimoto and Carneiro, 2001). However, development of larval feeds for raising *Pseudoplatystoma* sp. in captivity is critical to resolve the problem of providing natural food replacement for these fishes and developing domesticated stock that relies on formulated diets. Larval diets, when used as the sole source of nutrition, need to be produced from high-quality ingredients. When larval diets are used as supplementary feeds in pond culture, where zooplankton or other live feeds are available, they may also include some locally available ingredients. In addition, our research demonstrated that some "local" ingredients from Peru may have "growth enhancing effects" when supplemented in larval/juvenile fish diets (Dabrowski et al., 2003; Lee et al., 2004). Therefore, the larval diets that we will formulate for the proposed research include both high-quality ingredients and "local" products (maca meal; produced from a plant, *Lepidium meyenii*, growing in Peruvian Andes). Our proposed study could promote interests in developing further aquaculture of these indigenous species of Amazonian fish by providing information on its nutritional requirements.

Quantified Anticipated Benefits

The proposed study aims to investigate key aspects of reproduction and larval nutrition of *Pseudoplatystoma* sp. in order to improve or develop sustainable aquaculture technology for these species. Through collaborative efforts with Peruvian investigators and Brazilian collaborator, we will be able (1) to develop breeding techniques for *Pseudoplatystoma* sp. and (2) to develop methods of rearing first-feeding *Pseudoplatystoma* sp. larvae. The main beneficiaries of this research will be the local producers in the Peruvian and Colombian Amazon and neighboring countries (Brazil, Ecuador, and Bolivia).

The Amazonian catfishes are cultured in mono- or polyculture systems in order to control populations of the native cichlids, such as *Cichlasoma* and *Aequidens*. *Pseudoplatystoma* sp. reach large sizes (20-100 kg) and have a wide distribution throughout the Amazonian basin in Peru, Colombia, Venezuela, Brazil, so experience gained with these species will be applicable in many countries of the region. *P. tigrinum* is an attractive species for the aquarium business, so development of aquaculture technology of seed production in captivity will reduce pressure on natural stocks and create an additional source of income for local fish farmers.

This study will also contribute towards institutional strengthening by providing training for faculty and graduate students from San Marcos National University, and staff from IIAP, SINCHI, IMANI and CORPOAMAZONIA in Iquitos, on various aspects of fish reproduction and nutrition. Development of methods that will increase the quality of progenies of *Pseudoplatystoma* sp. will contribute significantly to the artificial propagation techniques of these Amazonian fishes.

Research Design or Activity Plan

Location of Work: Laboratory experiments on broodstock reproduction and weaning *Pseudoplatystoma* sp. to formulated diets will occur in The Ohio State University Aquaculture Laboratory, Columbus, Ohio.

Methods:

Objective 1. Determination of Gametogenesis and Gonad Differentiation in a Captive Stock of South American Catfish, *Pseudoplatystoma* sp.

Three-year old broodstock of South American catfish *Pseudoplatystoma* sp. have been raised since March, 2003 in our tropical aquaculture unit at The Ohio State University, Columbus, Ohio. Fish (48 individuals) are maintained in 200-L tanks at 25-30°C located in the greenhouse of the Department of Plant Biology and fed commercial feeds (BioDiet Brood, Bio-Oregon, Inc., 5 mm) at 1-2% body weight. Fish are individually tagged (Passive Integrated Transponder tag, PIT-Tag, Biomark, Inc., Boise, Idaho) and are periodically measured and weighed. The number of fish available (48) precludes extensive sampling for histology. However, we will collect samples in the proposed studies (including December 2004) 3 times a year and have representative set of samples to be analyzed. We will perform gonad biopsies and these samples will be analyzed for germinal vesicle migration (GVM) (Serra's solution) and by histological methods. Fish (at least 3 fish from each sex) will be periodically sacrificed, their gonads excised and fixed in Bouin's solution for further histological analysis during the first year of study. Gonads fixed 24 h in Bouin's solution will be then preserved in 70% ethyl alcohol until further processing. The tissues will be dehydrated in a series of ethyl alcohol and xylene baths and embedded in paraffin. Thin sections (5 μm) will be cut, mounted on albumin-coated slides, stained in Mayer's haematoxylin and eosin, and examined by light microscopy (Rinchard et al., 2002a).

Objective 2. Determination of Changes in Plasma Sex Steroid Hormones During an Annual Reproductive Cycle in *Pseudoplatystoma* sp.

Blood will be collected every 3-6 months from the caudal vessel using heparinized syringes. Blood will be centrifuged at 1,500 g for 15 min and the plasma stored at -20^{o} C until steroid assays. Fish maturity will be checked periodically. The plasma concentrations of steroids (testosterone, estradiol-17β, 11-ketotestosterone, and 17,20β-dihydroxy-4-pregnen-3-one) will be measured using radioimmunoassay similar to those used previously (Ottobre et al., 1989) following ethyl-ether extraction. Validation of these steroid assays has already been accomplished in our laboratory for other species such as rainbow trout *Oncorhynchus mykiss*, yellow perch *Perca flavescens*, and pacu *Piaractus* sp. (Dabrowski et al., 1995, 2003; Ciereszko et al., 1997, Rinchard et al., 2002b). Assays for these four steroids will be validated in a similarly rigorous fashion for the *Pseudoplatystoma* sp. Approximately 100 blood samples will be analyzed. 17,20-progestin will be only analyzed prior to ovulation or spermiation.

Objective 3. Induction of Reproduction of *Pseudoplatystoma* sp. and Assessment of Blood Plasma Steroid Response and Gamete Production (Egg and Sperm Quality)

If the fish are maturing, artificial reproduction with or without the use of hormone will be performed. Ovarian biopsies will be included in assessing response to hormonal stimulation. We have used this techniques previously (Dabrowski et al., 2003) in propagation of paku (*Piaractus brachypomus*). Both genders will be injected with two doses of carp pituitary extracts (5 mg/kg female; 1.5 mg/kg male) (Leonardo, 2003; Leonardo et al., 2004) or luteinizing hormone-releasing hormone analogue (LHRHa) (Dabrowski et al., 2003) to induce ovulation and spermiation. In the past, we had an immune inflammatory response to some preparations of of hCG (protein) (walleye, sauger) and had better success rates with releasing hormones. Therefore, it may be safe to use LHRHa when working with a “new” species. During the prescreening process already implemented in our procedures, we documented sex and advancement of maturation in individual (tagged) fish (Dabrowski et al., 2005. WAS, New Orleans) based on steroid profiles, and therefore sampling may be limited to females biopsies. The period of blood sampling will be changed to 3-4 month intervals in order to closely monitor events related to gametogenesis.

If the number of broodstock fish is sufficient, we will implement the standardized procedure described earlier in evaluation of gametes quality in rainbow trout, eggs and sperm separately (see Ciereszko and Dabrowski, 1995; Blom and Dabrowski, 1995). We have already an experience with *Pseudoplatystoma* at OSU facility in Columbus that hormonally induced males are providing copious amounts of sperm during prolonged periods (as it is the case in salmonids or percids). We will monitor the motility (duration and percent activation) of sperm prior to fertilization tests.

Sperm and ovulated eggs will be collected by stripping anesthetized fish. Eggs from each female will be fertilized with semen from three to four individual males and incubated in 9 cm diameter Petri dishes as well as in McDonald jars. Survival rates will be assessed at 1-2 hours (blastula) and at hatching (20 hours) (Cardoso et al., 1995; Kossowski, 1996).

Objective 4. Development of Larval Diets for *Pseudoplatystoma* sp.

Larvae obtained from fish induced to spawn by hormonal injections in our laboratory or alternatively, larvae obtained from Aquaculture Center, Sao Paulo State University, Jaboticabal (Brazil) will be used. All required documentation will be completed (Fish and Wildlife Service permit, Ohio Department of Natural Resources permit, import permit, health certificate from Brazilian authority) prior to importation of larvae of exotic fish species to the United States. M.C. Portella, our Brazilian collaborator will be involved in planning the experiments to be carried out in Columbus. In addition, parallel studies with the same batches of fish will be carried out in Jaboticabal, to ensure viability of larvae, feed acceptance and growth rates. There are several aspects of surubim (*Pseudoplatystoma fasciatum*) larvae behavior that need to be considered during rearing because of very high cannibalism in this species. The information on frequency of feeding, feed particle size, live food availability, illumination of rearing tanks will be provided by our collaborator, Portella.

The feeding experiment will be conducted in a flow-through system consisting of 24 aquaria (3 tanks/dietary treatment) supplied with aeration. Water quality will be monitored throughout the larval rearing process. Temperature (26-28^{o} C) and dissolved oxygen (5-6 mg/L) will be determined on a daily basis with weekly measurements of total ammonia-nitrogen and pH. Two days after yolk resorption, catfish larvae will be randomly distributed at a density of 100 larvae/aquarium and fed at a restricted ration up to 90% satiation for 2-4 weeks. Alternatively, live *Artemia* nauplii will be offered as initial food and then 7 or 14 day old larvae/juveniles will be weaned to formulated diets. An automatic feed dispenser will be installed (Charlon and Bergot, 1986) to deliver formulated diets. At the beginning of the experiment, samples of 10 larvae will be weighed. Larvae will be fed 5 diets: (1) a commercial diet (Kyowa Hakko Kogyo Co., Ltd., Japan), (2) an experimental casein-gelatin based diet with maca meal as attractant (Lee et al., 2004), (3) an experimental diet based on freeze-dried preparation of fish muscle (Hamre et al. 2001), (4) freshly hatched brine shrimp nauplii (Argent Chemical Laboratories, Redmond, WA), and (5) a marine diet (AGLONORSE, Ewos AS, Bergen, Norway). Both experimental diets will be formulated based on our previous experience (Lee et al., 2004) and will be isonitrogenous (protein requirement: 55% for most larval fish, Dabrowski, 1986). Larval samples (n=10) will be taken every week from each tank and fixed in buffered formalin for biometric measurements. At the end of the experiment, growth performance will be evaluated in terms of final individual body weight, survival (%), specific growth rate (SGR, %) and weight gain (%). Fish from each dietary treatment will also be sampled for proximate body analysis (water, protein, lipid, ash) if the size at the termination of the rearing period will permit (at least 0.5 g individual weight).

Statistical Analysis:

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

Regional and Global Integration

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

Schedule

Objectives 1 and 2:

September 2004 to March 2006: Evaluation of gonad development and assessment of plasma sex steroid hormone concentrations. Histological and steroid analyses.

March 2003 to May 2006: Synthesis of data and preparation of manuscripts and fact sheets and final reports.

Objective 3:

January 2005 to March 2005: Induction of spawning if maturity is reached. Spawning and evaluation of progenies (larvae data collection).

January 2006 to March 2006: Induction of spawning if not accomplished previously. Spawning and evaluation of progenies (larvae data collection).

March 2003 to May 2006: Synthesis of data and preparation of manuscripts and fact sheets and final reports.

Objective 4:
December 2005 and January 2006: Formulation and preparation of diets. Preparation of larval rearing aquaria.
February 2006: Collection of larvae and transfer to aquaria.
February 2006-April 2006: Feeding experiments for 6-8 weeks with larvae
April-May 2006: Collection of data on growth and survival, synthesis of data and preparation of manuscripts and fact sheets and final reports.

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Pond Design and Watershed Analyses Training

Water Quality and Availability 1 (11.5WQA1)/Activity/Honduras

Investigators

E. William Tollner	US Principal Investigator	University of Georgia
Dan Meyer	HC Principal Investigator	Panamerican Agriculture School, Honduras
George Pilz	HC Principal Investigator	Panamerican Agriculture School, Honduras

Objectives

1) Conduct training in pond design and watershed analysis for technical staff and managers in Central American nongovernmental organizations and governmental resource management agencies.

Significance

The hillsides of Latin America cover about 1 million km² and provide livelihood for some 20 million people, among whom roughly half are classified as "poor" and live in marginalized, rural communities (Knapp et al., 1997). Principal Central American countries (followed by % area in steep-slope agriculture) are: Honduras and Nicaragua (80%), Costa Rica (70%), and El Salvador and Guatemala (75%) (CIAT, 1996). Typically, the hilly landscape is very heterogeneous and made up of small plots. About half of the hillsides ecosystem in Latin America is progressively deteriorating due to the combined effects of deforestation, overgrazing, destructive tillage techniques, improper water management and unfavorable socioeconomic conditions (Whiteford and Ferguson, 1991; Knapp et al., 1997). This has serious implications for agroecological sustainability.

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

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

Quantified Anticipated Benefits

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

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

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

Research Design

Location of Work: The fieldwork for this activity will occur in a representative hillside micro-watershed in the Yeguare drainage basin of Honduras. Efforts will be made to expand on current training to include Panama and a site in Mexico. A major portion of the work will, however, be undertaken at Zamorano and at UGA campuses where additional facilities and expertise is available. The NetMeeting Software would be evaluated in a lab at Zamorano and, as experience was accumulated, migrate outward to other Central American countries, including Panama and possibly Mexico.

Methods:

Identification of Test Watersheds and Climatological Data: The first task will be to identify a micro-watershed in several selected Central American countries that typifies hillside regions and has existing fish farmers, including operators who use land and water resources for a range of agriculture-related activities. This has been completed in Honduras. A team of UGA and Zamorano personnel will do watershed identification.

Analysis and Modeling: This task will involve use of data and analysis tools to further assess the design criteria and identify suitable pond locations in the possible areas. In terms of spatial datasets, CIAT has developed a detailed atlas for several Central American countries that includes the latest datasets including an updated soils map as well as a digital terrain model (DTM) at the 50m-resolution level. These datasets together with climatic data provide a useful foundation for the modeling work. Hydrologic data from CIAT will be assessed via existing UGA contacts there.

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

Documentation and Delivery of Pond Designs: The pond designs developed will build upon past discussions with farmers in Honduras and neighboring countries with the current work plan. In collaboration with Zamorano and other institutions, we will also pursue the possibility of seeking resources to build one or more ponds provided all parties are agreeable. Workshops and training sessions are the primary delivery approaches. Copies of all handouts will be available in Spanish for workshop attendees. We anticipate two training sessions per year in Honduras, Panama and Guatemala. Each training session would involve 20 local NGO reps, university faculty and interested farmers.

Information Technology Transfer: A two-prong approach is envisioned for technology transfer. We envision the conversion of the Excel-based models to an appropriate web-enabled format. Some discussion of this was completed this past year when Jennifer Maldonado was in Honduras for training sessions this past spring. We propose to bring their IT person to Georgia for a seminar in Advanced Web delivery methods.

The other potential approach is to the use of Microsoft NetMeeting® or NetSupport software. This technology has been implemented in teaching Labs at UGA and can conceivably be implemented in teaching labs in Zamorano and other Central American locations. This (and all other software) requires knowledgeable IT support at each training site. These software packages are currently in use at the BAE department in Athens, Georgia. They would then implement the appropriate software package at their environment. This activity would be integrated with the cross cutting thrust in this project to enhance information delivery to in-country trainers via the Internet.

Regional Integration

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

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

Schedule

Work will commence October 2004. Initial tasks (identification of watersheds, documentation of linkages, and development of design criteria) will be completed by July 2005. The remaining tasks will be completed by June 2006.

Report Submission: As was done for the previous work plan, copies of the models with user guides were made available at training sessions. These models require numerous inputs regarding weather, land use practices and soils data, all of which are available are available in CIAT's GIS atlas for Central American countries. A report spanning the two years of this activity is planned to be submitted to the Aquaculture CRSP by 30 June 2006.

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

Water Quality and Availability 2 (11.5WQA2)/Experiment/Mexico

Investigators

Carl B. Schreck	US Principal Investigator	Oregon State University
Guillermo R. Giannico	US Principal Investigator	Oregon State University
Wilfrido M. Contreras-Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco
Gabriel Márquez-Couturier	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco
Ulises Hernández-Vidal	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco

Objectives

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

Significance

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

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

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

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

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

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

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

In our latest experiments (Schreck et al., 2004), we found that UV treatment of water resulted in higher masculinization rates of fry that were fed MT and that fry exposed to effluents from these systems were also masculinized. MT, however, was not detectable in a majority of the masculinization and effluent water samples. Masculinization of fish from the experiments indicated that MT (or a decomposition product not detectable by our RIA) was present in sufficient quantities to induce sex reversal. We speculate that MT may be present at levels that are below the detection limits of our assay but accumulate in fish over time resulting in masculinization. In the current proposal, we will sample and extract larger volumes of water to ensure detection of MT. If MT is still undetectable, further research will be needed to investigate the presence of possible MT metabolites which are not recognized by our assay.

Quantified Anticipated Benefits
The development of clean technologies for aquacultural practices will positively impact the production of sex-reversed tilapia fry. The use of UV sterilizers in intensive systems for masculinizing tilapia will improve safety in handling masculinizing steroids, produce clean effluents, and potentially increase efficiency of masculinization.

Research Design
Experiment 4. Elimination of MT from the Water of Intensive Sex-Inversion Systems at a Production Scale
Location: Experiments will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Oral administration of MT (dose = 60 mg/kg) in concrete tanks (8 m^2).

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

Culture Period: 3 months.

Stocking Rate: 2,500 fry/m^2.

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

Nutrient Inputs: None

Water Management: Water will be recirculated through 8000 l tanks equipped with biofilters. Exposure tanks will receive MT-treatment recirculated water; control tanks will receive untreated recirculated water. A 25% water exchange will be performed twice a week. Temperature, pH and dissolved oxygen, will be measured daily. Nitrates, nitrites and ammonia will be measured once a week, during the duration of trials.

Sampling Schedule: The experiment consists of three treatments, each treatment will be repeated three times:

- Fry fed control food for 28 days; water not recirculated through a UV sterlizer.
- Fry fed MT at 60 mg/kg food for 28 days; water not recirculated through a UV sterlizer.
- Fry fed MT at 60 mg/kg food for 28 days; water recirculated through a UV sterlizer.

Water and fish will be sampled and analyzed the same as in Experiment 2 except that a larger volume of water will be sampled (20 ml) and extracted with Sep-Pak cartridges to ensure that MT will be detectable by our RIA.

Statistical Methods and Hypothesis: H_o1: MT is not detectable in control water at any time. H_o2: MT is detectable in water at any time during treatment of tilapia fry with MT-impregnated food independently of the use of UV sterilizers. MT content of water will be compared between either control or treated water, UV exposed and unexposed, and for exposure time in treated water using ANOVA with $P < 0.05$. H_o3: Administration of MT-feed to tilapia held in systems with or without UV sterilizers produces fish with the same sex ratios as controls. Sex ratios will be compared by a Chi-square test.

Regional and Global Integration

If the results of the proposed study are successful, we will exchange information with Daniel Meyer, at the Pan-American Agricultural School. We have already discussed with the Peru and Thailand Aquaculture CRSP research teams our intentions to exchange information among project scientists and for potential technology transfer.

Schedule

Data collection, November 2004–June 2006; Final technical report, 30 June 2006.

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Elimination of Methyltestosterone from Intensive Masculinization systems: Use of Solar Irradiation and Bacterial Degradation

Water Quality and Availability 3 (11.5WQA3)/Experiment/Mexico

Investigators

Carl B. Schreck	US Principal Investigator	Oregon State University
Guillermo R. Giannico	US Principal Investigator	Oregon State University
Wilfrido M. Contreras Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco
Gabriel Márquez Couturier	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco
Ulises Hernández-Vidal	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco
Rosa Martha Padrón-López	Microbiologist	Universidad Juárez Autónoma de Tabasco

Objectives

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

Significance

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

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

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

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

It is well known that one of the major problems in aquaculture is the elimination of culture wastes from water. The amount and type of residues will depend on the species cultured, the stage of development and the feeds used (Wheaton, 1982). To lower the environmental impacts caused by aquaculture practices, different technologies have been developed to preserve water quality and reduce residue levels during fish culture. These systems are known as Recirculation Aquaculture Systems (RAS; Timmons, et al. 2001) and are widely used because they allow for efficient disposal of wastes in aquaculture.

In a previous investigation (10ER2) we developed an 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 though a perforated section of PVC pipe resulting in a "water curtain" which both aerates the water and exposes it to sunlight. Results from 10ER2 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 and passed through the RAS) also did not have detectable levels of MT.

In another investigation (11WQAR1) we demonstrated that exposure of MT treated water to sunlight resulted in reduced levels of the compound but it was not completely eliminated. Methyltestosterone is a light sensitive hormone which is subject to photodegradation (Budavari et al., 1989; Sigma Chemical Company, 1994). It is also known that some bacteria are capable of degrading steroids (see Mobus et al., 1997). 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.

It is also possible that MT is present in control water, but at very low concentrations which are below the detection limits of our assay. Even though the MT is not detectable it could be accumulating in fish over time. For this reason we will conduct two experiments. The first will use a large dose of MT (with no fish present) which will ensure detection by our radioimmunoassay (RIA). The second will simulate the daily pulses of low levels of MT seen during intensive masculinization of tilapia fry. A much larger water sample (20 times that of the first experiment) will be extracted to enhance the detection limits of the assay. This will allow us to determine if solar irradiation and/or bacterial degradation is capable of reducing or eliminating MT in a simulated feeding regime with no fish.

The goal of this investigation is to determine if bacteria are present within our RAS that are capable of degrading MT. The ultimate goal of our research will be to isolate, characterize and cultivate the species of bacteria responsible for degradation of steroids. Cultures of these bacteria could then be delivered to growers who could incorporate them into their filtration systems, thus eliminating the steroid in a completely natural way. Removal of MT could 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 for aquaculture systems.

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

Quantified Anticipated Benefits

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

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

Research Design

Experiment 1: Elimination of MT from the Water of Intensive Sex-Inversion Systems, Large Single Dose

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

Methods: Expose water containing MT to either sunlight, an RAS, or both.

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

Water Management: Water will be recirculated through 8000 l tanks equipped with RAS's with no activated charcoal or fish. Exposure tanks will receive MT-treated recirculated water; control tanks will receive untreated recirculated water. Water will be maintained at ambient temperature for exposure to sunlight and/or the RAS. Temperature, pH and dissolved oxygen, will be measured daily. Nitrates, nitrites and ammonia will be measured once a week, during the duration of trials.

Sampling Schedule: The experiment will consist of six treatments done in duplicate.

- MT treated water recirculated in the dark with no RAS (positive control).
- MT treated water exposed to sunlight with no RAS.
- MT treated water passed through the RAS in the dark.
- MT treated water exposed to sunlight and passed through the RAS.
- control water recirculated in the dark with no RAS.
- control water exposed to sunlight and passed through the RAS.

All exposures will be for 10 days. Water samples will be collected at 0, 2, 4, and 8 hours on day 1 and then once daily for the remainder of the experiment. Since it is possible that MT is being trapped in the sediments within the RAS (but not degraded), sediment samples will also be collected from various parts of the RAS including; plastic grate pieces in the sediment trap, the grate in the charcoal filter section, the synthetic fiber and plastic grate on top of the biological filter section, and from the pieces of plastic grate within the biological filter. Sediment samples will be collected at the same time as water samples.

Water samples (1 ml) and sediment samples (1 g) will be extracted with ether and MT content determined by RIA. Experimental water will be treated with MT at 100 mg $l^{-1}$and exposed to sunlight and/or passed through the RAS in the same manner as for control water. This is a dose that will ensure detection of MT in our assay during the early phases of the experiment.

Statistical Methods and Hypotheses: H_o1: MT is not detectable in control water at any time. H_o2: MT is detectable in MT treated water and in sediments within the RAS at all sampling times and independently of exposure to sunlight and/or the RAS. MT content of water will be compared between either control or treated water, exposure to sunlight and/or the RAS, and for exposure time in treated water using ANOVA with $P < 0.05$.

Schedule:

Data collection, November 2004–June 2006; Final technical report, 30 June 2006.

Experiment 2: Elimination of MT from the Water of Intensive Sex-Inversion Systems, Simulated Feeding

Regime
Location: Experiments will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, Mexico.

Methods: Expose water containing MT to sunlight and the RAS.

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

Water Management: Water will be recirculated through 8000 l tanks equipped with RAS's with no activated charcoal or fish. Exposure tanks will receive MT-treated recirculated water; control tanks will receive untreated recirculated water. Water will be maintained at ambient temperature for exposure to sunlight and the RAS. Temperature, pH and dissolved oxygen, will be measured daily. Nitrates, nitrites and ammonia will be measured once a week, during the duration of trials.

Sampling Schedule: The experiment will consist of two treatments done in duplicate.
- MT treated water exposed to sunlight and passed through the RAS.
- control water exposed to sunlight and passed through the RAS.

All exposures will be for 28 days. Water samples (20 ml) will be collected daily and extracted with Sep-Pak cartridges and MT content determined by RIA. Experimental water will be treated daily with MT at a dose that is comparable to what the system would receive if fish were present. Water will be exposed to sunlight and passed through the RAS in the same manner as for control water.

Statistical Methods and Hypotheses: H_o1: MT is not detectable in control water at any time. H_o2: MT is detectable in MT treated water at all sampling times. MT content of water will be compared between either control or treated water, and for exposure time in treated water using ANOVA with $P < 0.05$.

Schedule:
Data collection, November 2004–June 2006; Final technical report, 30 June 2006.

Regional and Global Integration
If the results of the proposed study are successful, we will exchange information with Daniel Meyer, at the Pan-American Agricultural School. We have already discussed with the Peru and Thailand Aquaculture CRSP research teams our intentions to exchange information among project scientists and for potential technology transfer.

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Ecological Assessment of Selected Sub-Watersheds of the Nzoia River Basin

Water Quality and Availability 4 (11.5WQA4)/Study/Kenya

Investigators

E. W. Tollner	US Principal Investigator	University of Georgia
Mucai Muchiri	HC Principal Investigator	Department of Fisheries, Moi University
Odipo Osano	HC Principal Investigator	School of Environmental Studies, Moi University
Geoffrey Karanja	HC Principal Investigator	Department of Wildlife Management, Moi University
William Shivoga	HC Principal Investigator	Department of Environmental Studies, Egerton University

Objectives

1) Assess aquatic ecological health of selected representative sub-watersheds by analyzing biotic habitat characteristics.
2) Establish fish and macroinvertebrate population characteristics.
3) Analyze ecotoxicological characteristics of representative biota.
4) Relate land use to habitat and biotic assemblages characteristics.
5) Develop a model for rapid assessment of watershed ecological health by use of biological indicators.

Significance

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

Protocols for monitoring biophysical characteristics of the landscape conditions at sampling sites will be developed throughout the watershed in order to capture the spatial distribution of landscape conditions as a function of biophysical and anthropogenic activities. These protocols will identify key indicator species, which must be identified for each physiographic region. Resources such as Jensen and Bourgeron (2001) and IoEA (1995) provide excellent guidelines for baseline determination and subsequent survey approaches. They discuss methods for prioritization of resources and time to achieve results appropriate to local and regional scales. Ludwig and Reynolds (1988) discuss techniques such as canonical correlation that are useful for exploring connections between ecological indicators and environmental variables that can be tailored to watershed assessment objectives. Excellent in-country resources exist for ecological assessment (e.g., http://www.iaia.org/Members/Publications/Guidelines_Principles; and http://www.kws.org/kwstidiploma.htm).

Both rural and urban land uses within watersheds invariably have effects on biodiversity. This includes terrestrial and aquatic biota. Habitats are altered leading to variation in biotic population structures. In streams the effects come in the form of variation in water quantity and quality. Land uses generate both organic and inorganic pollutants that alter the physicochemical quality of the water. Such altered water characteristics in turn influence changes in biological communities. Pollutants entering a river system at identifiable points are often evaluated using physical and chemical measurement techniques. However, in certain situations, particularly in rural agricultural areas, pollutant sources are more diffuse and thus lending it difficult to make direct measurements. Fish and macroinvertebrate communities are good indicators of ecosystems quality as the kinds and abundances of animals will vary according to a wide variety of physical habitat differences such as habitat size, temperature, stream flow or water depth, and pollution. The present surveys will aim at describing a) the community structure, and b) community processes and interaction for both fish and macroinvertebrates throughout the Nzoia River basin. The cause – impact relationships of land use and biological communities have been used fairly successfully in Europe and the United States in diagnosing ecological health of watersheds. This has been done based on identifying a portfolio of impact indicator species.

Anticipated Benefits
It is anticipated that by using ecological characterization techniques, appropriate species for assessment will be identified and results will be used to develop a rapid assessment model that can be used to rapidly indicate the ecological health of the selected sub-watersheds and by extension the whole of the Nzoia River basin. In this project, fish and macroinvertebrate assemblage will be used as biological "signatures" for aquatic health diagnosis. In addition, habitat characteristics will be used for both aquatic and terrestrial ecosystem health analysis.

Research Design
The Nzoia basin contains a variety of geomorphic formations, ranging from pristine fast moving streams, wetlands, reservoirs and discharge into Lake Victoria. Selected stations will be characterized for selected indicator species of river health. At least one existing industry (sugar processor or paper mill), an upland wetland and reservoir will be assessed for species composition and diversity. These will be coordinated with water quantity/quality sampling expeditions. As with the hydrology and water quality objective, emphasis will be directed toward developing the capacity of Moi University in the course of accomplishing these goals. The initial goals will be developed with close cooperation and with coordination of goals from nearby watersheds such as the Njoro that are currently being studied. Over the life of this project, initial ecological characterization of these potentially sensitive areas is the expected outcome.

Proposed Methodology: The Nzoia basin fish and macroinvertebrate biodiversity surveys will be based at Moi University and one of the Mt. Elgon Park camp sites near the park headquarters.
For the most part, choice of sampling points will be determined by perceived impacts of the various land uses. In forested areas sampling stations will be influenced by accessibility on motorable roads. However, every effort will be made to access as many habitat types as possible even on foot where safety from wildlife permits. On reservoirs and the mouth of Lake Victoria a rubber dingy will be used to access the open water.

Collection:

Fish:

Sampling of fish will rely on several collection gear. Seine nets will be used in clear shallow banks, gill nets will be used in lagoons and other open water habitats while electric fishing apparatus will be used in habitats that netting would be difficult. A fry seine, a dip net and traps will also be used where appropriate.

Handling of fish after capture will be aimed at:

1) identifying, photographing, fixing and preserving representative specimens of all the species present in all the water bodies in the sampling areas; and
2) describing population structures and basic biology and ecology of the species present.

Participants in the Fish Surveys: To achieve the desired results, the following institutions will be involved in the survey:

- Moi University (MU-FISH) (Dr. Muchiri) will co-ordinate the fish ecology exercises.
- The Department of Fisheries, Ministry of Agriculture (FD-GoK).
- Personnel of Kenya Wildlife Service (KWS) based at Mt. Elgon National Park will participate in the field exercises where appropriate and will also be requested to provide security within the park.

Macroinvertebrates

Macro-invertebrate collection in the river and its tributary streams will employ a Surber Sampler and D-frame net Sampler in different habitat types pre-identified along the selected parts of Nzoia River. In Lake Victoria and reservoirs within the basin, an Ekman grab will be used to collect bottom invertebrates. A hand net will also be used to collect invertebrates in vegetated areas.

Samples collected will be preserved to be later identified and counted in the laboratory. This survey is aimed at describing population structures and basic biology and ecology of the species present.

To achieve the desired results, the following institutions will be involved in the macroinvertebrate survey:

- Moi University
- Egerton University (Dr. Shivoga) will co-ordinate the macro-invertebrate ecology exercises.

Personnel of Kenya Wildlife Service (KWS) based at Mt. Elgon National Park will participate in the field exercises where appropriate and will also be requested to provide security within the park.

Ecotoxicological Studies: Representative samples from the fish and invertebrate surveys will be preserved for ecotoxicological analyses. Laboratory analyses will focus on heavy metals and pesticide accumulation and effects on fish and macroinvertebrates. These studies will be coordinated by Moi University (Dr. Osano). Appropriate statistical designs and analyses will be employed in the ecological studies.

Statistical Design, Null Hypothesis, and Statistical Analyses: Fish and macroinvertebrate surveys will be collected using locations along the Nzoia (or tributaries) as reference locations. Key location attributes will be 1) distance from outlet; 2) water quality attributes; 3) key hydrologic descriptors and 4) season. The null hypothesis is that no differences in selected species exist across location. ANOVA techniques (Hintze, 2004) and paired t tests will be used as appropriate.

Regional Integration

The project is targeting the development of a watershed and basin assessment center at Moi University by building a physical science and social science interdisciplinary center to complement such centers at Egerton and Nairobi universities. This project is inherently integrative as it initiates and increases cooperation among diverse interests with the common thread of maintaining the health of a common benefit, the community river. The concept is replicable in other parts of Kenya as well as other developing countries. Close working collaboration will be maintained with the Sustainable Management of Watershed – CRSP (SUMAWA) project that studying the River Njoro watershed. The concept is replicable in other parts of Kenya as well as other tropical regions.

Schedule

Review of historical data and literature on ecological characteristics of the Nzoia River basin (Jan 2005–June 2005).

Field surveys are expected to take place between January and December 2005. Sampling will be carried out once every month for nine months. This will cover both the short and long rains periods. Each field sampling expedition will last four days.

Periodic reports of progress will be made in the course of the ecological surveys.

Final data analyses and reporting (October 2005–December 2005).

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Determination of Hydrologic Baselines for the Nzoia Basin

Water Quality and Availability 5 (11.5WQA5)/Study/Kenya

Investigators

Mucai Muchiri	HC Principal Investigator	Moi University
Nancy Gitonga	HC Principal Investigator	Kenya Dept. Fisheries
Ernest W. Tollner	US Principal Investigator	University of Georgia
H. Farrah	HC Collaborator	Moi University

Objectives

1) Physical condition classification of watershed.
    - Map reconnaissance
    - Ecological assessment
2) Evaluate the potential impact of streamside cultivation on water quality parameters.
    - Improved riparian cultivation approaches based on computer modeling studies
    - Soil transport in on-the-ground studies
3) Water quality profile of the Nzoia .
    - Winter conditions
    - Summer conditions
4) Organize data to incorporate it into the GIS database.

Significance

The degradation of the Lake Victoria basin has received international attention (GEF, 2004). The 184,400 km2 basin drains 14 major rivers with an estimated population of 25 million people (Wangila and Swallow, 2001). The Kenyan portion of the basin drains 42,000 km2 with a population of 7.9 million people (Wangila and Swallow, 2001). The Nzoia watershed comprises 12,000 km2 of the Lake Victoria basin (United States Geological Survey, 2004). Four main processes drive the degradation of the Lake Victoria basin: 1) loss of 89% of forest cover to poor agricultural practices causing erosion and sedimentation; 2) pollution from mines, urban areas and industry leading to sedimentation in eutrophication; 3) loss of lake fish species diversity due to introduction of the non-native Nile perch; and 4) poor fisheries management practices (Wangila and Swallow, 2001). Sedimentation increased fourfold in the last 100 years with some areas demonstrating losses of 200 horizontal feet each year (GEF, 2004).

Kenya is on the verge of substantial agricultural development (GEF, 2004) and urbanization especially in the Nzoia basin (Osano et al., 2003). A search of the literature reveals that virtually every agricultural enterprise from forestry production to container nursery production has specific Best Management Practices (BMPs) for water quality management with nonpoint source inputs. The NRCS has an exhaustive array of BMPs available for agricultural production (NRCS, 2004) nonpoint sources. BMPs have recently been published for urban storm water management (WEF, 1998). Except for specific cases not involving soil tillage (e.g., container nursery in greenhouses), sediment is the most significant pollutant. Sediments are significant in both urban and rural development because a host of chemical constituents adhere to sediment particles. BMPs for urban and rural environments focus primarily on sediment removal and handling. Increased urbanization and increased intensity of agricultural production results in increased magnitude and frequency of runoff events, reduction of base flow and increased stream velocities when flowing (WEF, 1998). These flow changes lead to increased cross sectional areas, significant down cutting (unless stream is already heavily armored), increased sediment loads due to bank erosion, urban construction or intensifying agricultural production, modification of streambed to include more fine particles and subsequent stream modifications being required to reduce flooding risks. Increased urbanization and agricultural development affect water quality as well. Urbanization causes an initial pulse of sediment which subsides as the development stabilizes. Increased agricultural production increases pesticide (Osana et al., 2003) and sediment loads which may remain high, depending on the degree of soil tillage. The sediment load and consequent increase in fines cause benthic ecology to become much less diverse. Streams generally shift from an external (leaf matter) to an internal (algal organic matter) food chain. The stream community loses diversity and wetlands, springs and riparian buffers are damaged or lost due to excessive sediment, toxic compounds or both. The effects on receiving bodies (e.g., Lake Victoria in this case) are felt over longer time frames. After visible refuse and damage to aesthetics, nutrient enrichment and the resulting increase in primary productivity is the most visible sign of development. Lakes act as sinks for sediment-laden materials and take longer to recover from contamination than do streams. Heavy metal absorption, sediment deposition patterns near the outlet, increased algae production in the lake (which indicates possible eutrophication that can in turn lead to fish kills), loss of desirable species and increased “trash” fish species are documented to occur with development. These documented trends will be pivotal in prioritizing the work on the Nzoia basin. It is not too late to avoid these effects in the Nzoia basin.

The Nzoia basin is in the initial phases of development. Much cultivation in rural areas is conducted by women of the families on soils at the river edge. Environmental legislation on the books provides 30 m buffers; however the exact point of measurement of the buffers is legally unclear and thus there is effectively not an enforced buffer. A buffer design is needed that will preserve some of the existing benefits of being near the river while achieving water quality goals. Environmental quality within and around the Lake Victoria basin, the location of this projected intervention, is closely linked to land use practices (GEF, 2004). Some of the land uses have resulted in serious degradation of ecological integrity and hydrologic processes within the watersheds. This is shown by the loss of biodiversity and habitats as well as altered hydrologic regimes (GEF, 2004). Consequently, the trend has resulted in declining livelihoods of the inhabitants. These factors have contributed to overall poverty in the region. With this background, there is a need to develop strategies and mechanisms to stabilize and rehabilitate the watersheds in the region. The proposed project will embark on a multidisciplinary approach to develop and demonstrate improved and integrated sustainable management of watershed resources at a watershed scale. The watershed assessment effort and subsequent demonstration projects will be coordinated with needs of the Kenyan Department of Fisheries in terms of fostering aquacultural enterprises along the river and preserving the Lake Victoria fishery. Overall, the proposed project will complement other efforts in the region in creating sustainable interdisciplinary, broad-based watershed rehabilitation models through technical, social and policy interventions in land use and natural resources management. The fisheries department can provide much helpful reference information to provide an objective standard by which improvements can be measured.

The Nzoia basin contains a variety of geomorphic formations, ranging from pristine fast moving stream, wetlands, lakes and discharge into Lake Victoria. Selected stations will be characterized for selected indicator species of river health. At least one existing industry (sugar processor or paper mill), an upland wetland and lakes will be assessed for species composition and diversity.

A watershed water resources assessment is the basis of determining the possibilities of water resource utilization, control and development. A proper assessment requires the determination of the sources, quantity and quality of water resources which In this study, the initial baseline data to assess the conditions in the watershed will be collected. First an inventory and mapping of the characteristics of the watershed will be undertaken. Next data collection stations will be established at key location for water quantity and quality measurements.

The US NRCS developed the Universal Soil Loss Equation (Wischmeier and Smith, 1978). The USLE predicts soil detachment. Soil delivered to some point below the erosion location may be predicted using a sediment delivery ratio. The US Forest Service (1980) published an approach for evaluating sediment delivery ratios through buffer strips. Coupling the NRCS soil loss equation with the Forest Service delivery ratio estimation approach enables the computer modeling of buffer strip scenarios that can prove efficacious for water quality preservation.

Anticipated Benefits

Perhaps the single most important variable in achieving sustainable watershed management is understanding and underpinning key land use practices that directly or indirectly affect ecological processes and system functioning. The reliance on land for agrarian production in rural Kenya coupled with dependence on land resources for economic livelihood places enormous premium on resources derived from land and as a consequence leads to degradation and hence loss of ecological integrity of the system (GEF, 2004; Githaiga et al., 2003; Osana et al., 2003). A sustainable land management strategy requires not only intervention at site specific but also the landscape level. A system approach is needed to disentangle critical landscape components and linkages and will more likely to lead to overall positive impacts on the watershed. The Nzoia watershed system transcends a broad range of land use systems and practices ranging from small-scale holder farmland to large scale mechanized agriculture, and cuts a cross a tenure regime of private ownership to public land – e.g., forest reserves and national parks. The watershed produces 30% of Kenya's maize and sugar (Osana et al., 2003). The watershed occurs in generally high potential and high population region of the country and therefore the influence of land use on the system is extremely important (Osana et al., 2003). Eldoret serves as the largest population center (234,000) followed by Kitale (88,100), Kakamega (86,500), Webuye (45,100), Mumias (36,200) and Bungoma (32,900) (Osana et al., 2003). Although agriculture comprises the major land use, textile, paper, sugar and coffee processing comprise major point source pollutants (Osana et al., 2003).

Given the character of the Nzoia watershed, the emphasis in this initial project will be to focus on BMPs for mitigating agricultural impacts. A desired outcome of this study is to develop a buffer design that will preserve some advantages of cultivation near the river while also providing water quality benefits.

Research Design

Objective 1. Physical Condition Classification of Watershed

The map reconnaissance will be completed as part of Activity 1. A physical condition checklist (Prichard et al., 1998, 1999) will be completed at some key locations to be coordinated with identified riparian buffer sites (see objective 2).

A thorough literature review will be conducted to gather data from previous hydrological studies and reports. Information on location of existing rainfall, river gauging and water quality measurement stations in the selected watershed will be obtained. Data from existing rainfall and river gauging stations will be obtained from Kenya Meteorological Department and Ministry of Water Resources Management and Development . Topographic maps of the watersheds at scales of 1:50,000 will be purchased at Survey of Kenya. Satellite data from Landsat TM will be acquired to prepare a land use and land cover map of the watershed. Soils and geology data will be obtained form Kenya Soil Surveys Department. All the data collected will be input in a GIS. GIS will be used to perform spatial and temporary data analysis and information extraction and improve the efficiency and quality of the watershed assessment.

Physical condition checklist results will be coordinated with water quantity/quality sampling expeditions. As with the hydrology and water quality objective, emphasis will be directed toward developing the capacity of Moi University in the course of accomplishing these goals. The initial goals will be developed with close cooperation and with coordination of goals from nearby watersheds such as the Njoro that are currently being studied using Participatory Rural Appraisal (PRA) in conjunction with the University of Wyoming, Egerton University, and Moi University. The Western Kenya Integrated
Ecosystem project also plans to use PRA to address watershed conservation in the Nzoia and two other watersheds (GEF, 2004). Over the life of this project, initial ecological characterization of these potentially sensitive areas is the expected outcome. These data would be integrated into the GIS database discussed in Activity One.

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

Protocols for monitoring biophysical characteristics of the landscape conditions at sampling sites will be developed throughout the watershed in order to capture the spatial distribution of landscape conditions as a function of biophysical and anthropogenic activities. These protocols will identify key indicator species, which must be identified for each physiographic region. Resources such as Barbour et al., (1999), Jensen and Bourgeron (2001), Sayer et al. (2000) and IoEA (1995) provide excellent guidelines for baseline determination and subsequent survey approaches. They discuss methods for prioritization of resources and time to achieve results appropriate to local and regional scales. Ludwig and Reynolds (1988) discuss techniques such as canonical correlation that are useful for exploring connections between ecological indicators and environmental variables that can be tailored to watershed assessment objectives. Excellent in-country resources exist for ecological assessment (e.g., http://www.iaia.org/Members/Publications/Guidelines_Principles; and http://www.kws.org/kwstidiploma.htm).

The first step in the assessment is to spatially segregate the area into manageable unit areas. The division into such units will be done systematically to define the watershed boundaries, hydrogeological, administrative and water management characteristics. Each unit will represent an area such that it is possible to collect and analyze the required data for purposes of water balance studies. The units should be able to be aggregated in a hierarchy manner to determine information at watershed, sub-basin, basin or regional level.

The number and location of data collection station in the representative sub-watersheds will be carefully selected, giving due consideration to the extent of the spatial and temporal variations of hydrometeorological and water quality factors. As a minimum, at least two data collection stations at the inflow and outflow points of the sub-watersheds will be needed. However, a number of other monitoring stations will be required. The criteria of locating the other stations are as follows:

- A gauging station should be located at each confluence of a tributary, if the flow of the main river is increased by more than 5% immediately after the confluence.
- A water withdrawal monitoring station should be located at each water withdrawal point if the amount of withdrawal is greater than 10% of the 95% probable low flows.
- A discharge station to monitor quantity and quality of water discharged into surface water from point sources, should be located at places where discharge is greater than 10% of the 95% probable low flow.
- A data collection station for quantity and quality monitoring should be located at the inlets and outlets of reservoirs, lakes and urban centers.

Other types of observations to be undertaken are the monitoring of the quantity and quality of water from springs, groundwater elevation and quality of groundwater resources at selected sites and the monitoring of meteorological parameters. Sediment loads in surface water will be investigated by installing sediment retention ponds at selected urban locations.

The temporal scale or frequency of monitoring the quality and quantity of water will be considered depending on the components of water resources (i.e surface or groundwater). All water quantity data will be collected on daily basis. However, modification may be necessary during actual study depending on the situation of the selected sub-watersheds.

Objective 2. Evaluate the Potential Impact of Streamside Cultivation on Water Quality Parameters

A computerized version of the USLE will be developed and coupled with the Forest Service sediment delivery ratio to predict relative soil loss from buffer configurations. The following buffer scenarios will be modeled:

- Forested buffer 30 m wide;
- Grass buffer 30 m wide;
- Completely disturbed buffer zone;
- Grass buffer with alternating 5 m cultivated strips and grass within 10 m of the river;

- Grass buffer with alternating 5 m cultivated strips and forest within 10 m of the river; and
- Forest buffer with alternating 5 m cultivated strips and forest within 10 m of the river.

Each scenario will be modeled with high, medium and low runoff conditions enveloping expected conditions to be found.

Two to three sites will be selected for riparian buffer studies. These sites will be in key geomorphologic regions (Mt. Elgon, Eldoret/Kitale plateau, Kakamega/Webuye lowlands) and will be coordinated with water quality assessment areas. Rainfall energy data may be estimated by correlating rainfall and erosion patterns in the US (where much is known) to Kenyan conditions using ILAC-OBV (1981) and Lal (1994). These reference give excellent worldwide trends and enable linking to similar zones in the US where much detailed climatological analyses results are available.

Soil texture analyses, slopes and slope lengths, cropping and conservation practices will be determined using Moi University lab and map recon as needed. The US PI will supply surveying equipment as needed and will supervise required surveys. Sites will be selected with uniform slopes comprising the respective field and riparian buffer conditions. Erosion will be monitored by the use of erosion stakes (see Tollner, 2002).

<u>Objective 3. Water Quality Profile of the Nzoia</u>

Stations along the Nzoia will be equipped with staff gauges and surveyed cross sections for water quantity measurements. Sites will be coordinated with the sites of Objective 2. Selected water quality measurements (such as N, P, BOD, turbidity) will also be taken (Githaiga et al., 2003) in winter and summer conditions in order to gauge the general water quality in a range of rainfall conditions. These data would be collected as part of Activity 1.

For water quality, groundwater quality may change relatively slowly while surface water quality changes much more rapidly. Moreover the quality of water discharge from point sources may change quickly and similarly water quality can change with time depending on the parameter being measured. In this study the quality parameter to be observed are N,P,BOD and turbidity. Groundwater and water from springs will be monitored on monthly basis while surface water will be monitored on weekly or bi-monthly basis.

The data collection, storage, analysis and presentation methodologies of the above mentioned monitoring scheme will follow already developed methodologies (eg UNSECO, 1988). It is assumed that the methodologies are adequate and can be implemented easily at the sub-watershed level.

<u>Objective 4. Organize Data to Incorporate It into the GIS Database</u>

This will be completed under Activity One.

Statistical Design, Null Hypothesis and Statistical Analyses: The sediment delivery model will be evaluated via sensitivity analyses by setting envelopes for rainfall, soil type, slope lengths, slopes, prevailing cropping practices and prevailing conservation practices to arrive at a distribution of expected soil loadings via the universal soil loss equation to which the buffer designs would be subjected. A worst case and best case of each parameter would be determined and modeled, requiring 25 or 64 erosion loadings. Each of the 64 erosion loadings would be evaluated with the 6 buffer designs. This would be repeated for each of the three regions of the Nzoia.

The null hypothesis, stated in a positive sense here, is that one or more of the designs will provide a reduction in soil loading equivalent to generally accepted soil loss tolerances for a field size typically found in the respective region. In other words, the goal is to use the buffer to enable soil conservation expected for good agricultural practices in the region.

The data will be analyzed following regression approaches and ANOVA approaches. Regression relations will be established for buffer strip widths with the forest and grass conditions. Soil losses from the 64 soil erosion runs will be grouped into three equal sized groups and mean soil loadings for the groups determined. This will be done by test region. These will form the basis for a randomized complete block ANOVA assessing buffer attributes. The emphasis of the analyses will be on relative benefits of the buffer.

Ideally, one could in a limited time verify the erosion models with field data. Three field sites will be established with buffers instrumented with erosion stakes at the beginning of the study. The stakes would be monitored at the conclusion of the study to determine the erosion/deposition documented at the individual stakes. Buffer strips would be instrumented with erosion stakes placed on a 10 m grid spacing. Demonstrations buffers 40 m long (length measured along the river) and 30 m wide would be established along side of comparable zones with cultivation next to the river. Sites would be selected to maximize usefulness for all project activities. Efficacy of buffers will be assessed in a relative sense at each location. The relative efficacy should correlate with the modeled efficacy (normalized to give relative efficacy). Initial results from the riparian buffer BMP study should be available at the project conclusion.

Regional Integration

This project is inherently integrative as it initiates and increases cooperation among diverse interests with the common thread of maintaining the health of a common benefit, the community river. The concept is replicable in other parts of Kenya as well as other developing countries. We will collaborate with other participatory watershed efforts such as those facilitated by the Kenya Agricultural Research Institute (KARI) through the Global Environmental Facility (GEF) and The World Bank, The International Centre for Research in Agroforestry (ICRAF), the Ministry of Agriculture and Rural Development (MoARD), and Egerton University (Wangila and Swallow 2001), as well as the Lake Victoria Improved Land Management Program. The Soil and Water Conservation Branch of the Ministry of Agriculture has implemented Participatory Rural Appraisal (PRA) in several watersheds in an ongoing basis (Thompson 1995). Colleagues at Michigan State University also co-direct the Land Use Change Impacts and Dynamics (LUCID) project in Kenya and Uganda in conjunction with the International Livestock Research Institute in Nairobi and the University of Nairobi. The LUCID project also addresses socioeconomic drivers of land use, gender dynamics, erosion and water quality.

Schedule

RESEARCH ACTIVITY PLANS Activity IV (Study)	Responsible Person(s)	Personnel	Timeline
Identification representative riparian sites in the regions of the Nzoia	Muchiri, Tollner	Gitonga, Odipo, Kiyiapi, Shivoga, Khamasi, Farah	March 2005
Erosion workshop and assessment workshop	Tollner	Team	March 2005
Computer modeling	Tollner	Graduate student	March-July 2005
Installation of gauging stations to define river morphology and install riparian buffer BMP at selected stations	Tollner, Farah	Karanja, 1 graduate student	July 2005
Monitoring of gauging stations	Tollner, Farah	Karanja, 1 graduate student	July 2005-Jan 2006
Organize data	Mucai, Farrah	Kenyan Team	Jan 2006

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Assessment of Coastal and Marine Aquaculture Development for Low Trophic Level Species

Economical Risk Assessment and Social Analysis 1 (11.5ERA1)/ Study / Global

Investigator

James Diana	US Principal Investigator	University of Michigan

Objectives

As part of a broader study on utilizing low trophic species for coastal and near shore aquaculture in developed and developing countries, this particular study focuses on identification of the strengths and weaknesses of low-trophic species for nearshore aquaculture from a progressive development framework.

Sub-Objectives

1) Address key development variables such as potential conflicts with other user groups, allocation of finite resources, organizational capacity, property and property rights, legality, and the post production harvest phases.
2) Investigate the potential ability of near shore aquaculture involving low trophic level species to function as part of integrated farming systems in coastal areas.
3) Investigate the economic, social and technical viability of culturing native species, in particular those at low trophic levels.
4) Determine benefits and costs to producing and/ or consuming societies.
5) Examine and evaluate case studies of current near shore aquaculture operations.

Significance

Though traditional near shore aquaculture has proven problematic in many locations, the farming of low trophic level species, especially bivalves, presents a potentially beneficial method of assisting near shore zones to process the influx of nutrient run-off from coastal aquaculture and agriculture practices. The hypoxic effects of nitrogen and phosphorous run-off can have a severe impact on the productivity and ecosystem functioning in near shore zones, which already are under intense pressure due to overuse. Integrated farming systems that utilize this region in order to increase overall harvest size, utilize wastes in a productive manner, and mitigate the effects of potentially detrimental land activities may present valuable economic and environmental opportunities to developed and developing countries alike. This study is advantageous because it will go beyond technical feasibility, and spend significant research time identifying salient environmental, legal, social, and economic concerns that remain unaddressed at present.

Anticipated Benefits

In part, we intend to address social and economic costs and benefits of different near shore production scenarios for low-trophic level species, as well as to compare the aquaculture with other competing sectors such as fishing, transport and tourism. By conceptualizing a broad interdisciplinary approach to this type of aquaculture, the promises and the pitfalls concerning future development can be anticipated.

Methods

This study is to be conducted largely through literature review, phone interviews, and case studies. This will be begn in January 2005 using the extensive University of Michigan library system, as well as electronic databases such as Aquatic Sciences and Fisheries Abstracts.

Regional and Global Integration

This study addresses a form of aquaculture prior to it becoming a widespread commercial production practice.

Schedule

Start data collection January 2005.
Final report 30 September 2006.

Hydraulic, Water Quality and Social Assessment of the Nzoia Basin, Kenya

Economic/Risk Assessment 2 (11.5ERA2)/Study/Kenya

Investigators

Geoffrey Habron	US Principal Investigator	Michigan State University
Mucai Muchiri	HC Principal Investigator	Moi University

Objectives

1) Develop stakeholder involvement model.
    - Actively engage stakeholders in the assessment of problems, needs and opportunities.
2) Promote communication between stakeholders.
    - Provide tools and information to stakeholders to enable participation in decision-making, planning, and rehabilitation activities.
3) Assess socioeconomic status of resource users to develop data sets to use in monitoring and evaluation.
4) Gender analysis in determining stakeholder involvement.
5) Organize existing social data at Moi University to incorporate it into GIS analysis.

Significance

The degradation of the Lake Victoria basin has received international attention (GEF, 2004). The 184,400 km2 basin drains 14 major rivers with an estimated population of 25 million people (Wangila and Swallow, 2001). The Kenyan portion of the basin drains 42,000 km2 with a population of 7.9 million people (Wangila and Swallow, 2001). The Nzoia watershed comprises 12,000 km2 of the Lake Victoria basin (United States Geological Survey, 2004). Four main processes drive the degradation of the Lake Victoria basin: 1) loss of 89% of forest cover to poor agricultural practices causing erosion and sedimentation; 2) pollution from mines, urban areas and industry leading to sedimentation in eutrophication; 3) loss of lake fish species diversity due to introduction of the non-native Nile perch; and 4) poor fisheries management practices (Wangila and Swallow, 2001). Sedimentation increased fourfold in the last 100 years with some areas demonstrating losses of 200 horizontal feet each year (GEF, 2004).

Kenya is on the verge of substantial agricultural development (GEF, 2004) and urbanization especially in the Nzoia basin (Osano et al., 2003). Perhaps the single most important variable in achieving sustainable watershed management is understanding and underpinning key land use practices that directly or indirectly affect ecological processes and system functioning. The reliance on land for agrarian production in rural Kenya coupled with dependence on land resources for economic livelihood places enormous premium on resources derived from land and as a consequence leads to degradation and hence loss of ecological integrity of the system (GEF, 2004; Githaiga et al., 2003; Osana et al., 2003). The Nzoia watershed system transcends a broad range of land use systems and practices ranging from small-scale holder farmland to large scale mechanized agriculture, and cuts a cross a tenure regime of private ownership to public land – e.g., forest reserves and national parks. The watershed produces 30% of Kenya's maize and sugar (Osana et al., 2003). The watershed occurs in generally high potential and high population region of the country and therefore the influence of land use on the system is extremely important (Osana et al., 2003). Eldoret serves as the largest population center (234,000) followed by Kitale (88,100), Kakamega (86,500), Webuye (45,100), Mumias (36,200) and Bungoma (32,900) (Osana et al., 2003). Although agriculture comprises the major land use, textile, paper, sugar and coffee processing comprise major point source pollutants (Osana et al., 2003).

Anticipated Benefits

We anticipate this project to lay the foundation for protecting the Nzoia basin from unsustainable development. Our use of participatory approaches will facilitate shared ownership of research projects, community-based analysis of social problems, and an orientation toward community action (Kemmis and McTaggart, 2000). This component will include the development of a model of stakeholder involvement to actively engage stakeholders in the process of problems/needs/opportunity assessment. To foster effective communication between stakeholders, policy makers, and scientists to make each aware of the others concerns and knowledge base(s), it is essential to engage stakeholders in the process of active and sustainable involvement. This in turn will provide stakeholders with the necessary knowledge to participate effectively in decision-making, planning, and rehabilitation activities. The pressing socioeconomic demands of increasing populations in watersheds tend to dictate the levels of resource use. Governments in many developing countries are unable to establish relevant policies to regulate use while maintaining the condition of the base resources. Consequently, the resource users have continued to exert pressure on the resources to provide for their basic needs at the detriment of the watersheds. Utilization of such resources often is unsustainable leading to the general impoverishment of the people and the environment. Among the various problems in the watershed, is the vicious cycle of poverty arising from intensified cultivation of riparian ecosystems as well as steep slopes at high altitudes without proper conservation structures. The intensification of socioeconomic activities in the watershed is suspected to be responsible for the loss of watershed integrity and observable loss of water quality and quantity. In view of the importance of human activities in the watersheds, there is need to assess the socioeconomic status of resource users in order to develop data sets that would be used for project monitoring and evaluation.

Research Design

Methods: Watersheds do not follow political boundaries often leading to difficulty in developing management plans. The Nzoia watershed spans the Western, Nyanza, and Rift Valley Provinces. Nzoia River Basin can be divided into three sections to include lowlands, upper plateau region and highlands. Kakamega and Webuye are the major towns in the lowlands where land cover consists of irrigated and rain fed herbaceous crops, tree and shrub crops and permanently flooded lands. Eldoret and Kitale are large towns found in the upper plateau where land cover consists of plantation and natural forests, rain fed crops, shrub savannah, and permanently flooded areas. The highlands are characterized by Mt. Elgon and the Cherangany hills where the land cover is dominated by several stages of forest cover. Given the diversity of land use activities within the watershed, it is important to capture the demographic, social, and resource management characteristics in each of the three regions when developing watershed management models.

Watershed stakeholders will we be assessed through a set of Participatory Learning and Activity (PLA) approaches to identify community demographics, gender roles, risk perceptions, and community social structure (Chambers, 1995). An initial identification of working groups, non-governmental organizations, cooperatives, government agencies, religious and family groups with an existing interest in water resource management will help identify starting points for further stakeholder interests. A review of past and current best management practices within the basin will help identify which groups of people have been targeted as watershed stakeholders and what their level of involvement is. These approaches acknowledge the importance that local communities have in “understanding the etiology, consequences, and nuances of complex problems” (Smith et al., 2000:1946). Participatory risk mapping demonstrates different risk perspectives across gender, class, location and level of agricultural activity among pastoralists sharing a semi-arid region of Ethiopia and Kenya (Smith et al., 2000). Previous Participatory Rural Appraisals (PRA) work in the Yala and Nyando basins, as well as others in Kenya (World Bank, Undated) can be used to compare watershed stakeholder involvement and the implementation of best management practices between the three basins. Further identification of watershed stakeholders and their involvement in decision making concerning land use and water use will be assessed through PRA. The data attained through PRA assessments combined with hydrologic conditions and natural hazards within the watershed are important in determining individuals’ perceived risks. An understanding of communities’ vulnerability to risk and their actions to prevent exposure will aid in the planning of resource management activities.

Women are centrally responsible for domestic water supplies, family health and hygiene, and carry out important roles in both farm and non-farm household income production activities that have implications for the sustainability of watershed resources. Furthermore, a larger portion of poorer households in rural and urban areas tends to be women-headed. For this reason, gender analysis will be included in the participatory rural appraisal methods to be used during the assessment phase with communities in laying the foundation for stakeholder involvement in managing the watershed (Kerr et al., 2002; Overseas Development Institute, 1999).

The work plan includes completing PRA assessments in each region. Each region will be subdivided into three sections; each of these sections will have separate PRA events based on gender (World Bank, 2004; Vainio-Mattila, 1999). Eighteen PRA assessments will be held across the watershed, with the possibility of holding focused PRA events for specific stakeholders within sections based on land use activities and land tenure. Data will be collected through observation, interviews and PRA events to include, transect walks, seasonal calendars, social mapping, wealth ranking, problem rankings, resource ranking/mapping, and chore delineation.

Timeline:
Literature review, identify current development projects in the watershed: January 2005–June 2005.
Data collection in Kenya July 2005–October 2005.
Lowlands (Kakamega and Webuye): 3 weeks.
Upper Plateau Region (Eldoret and Kitale) – 3 weeks.
Highlands (Mt. Elgon and the Cherangany hills) – 3 weeks.

Data Analysis: October 2005–December 2005.

Statistical Design, Null Hypothesis, and Statistical Analysis: We will compare responses across regions (3), subregion (3) and gender (2). We will utilize qualitative analysis using risk maps and risk priorities to investigate the following questions:

1) What are the perceived risks by watershed stakeholders? Who in the households and community are vulnerable to these risks?
2) Is household risk vulnerability determined by location, gender, and land tenure?
3) What role does gender play in household decision making to prevent exposure to risks?
4) What activities are people actively engaged in to reduce their risks? How do these activities affect natural resource management?

We will utilize participatory risk mapping (Stone, 2001; Smith et al., 2000) stratified by location and gender. Risk mapping proceeds first by soliciting community input regarding risk identification, and then facilitating the ranking of risk incidence and severity (Smith et al., 2000). Analysis designates incidence or frequency from a scale of zero if no one is affected to 1 if everyone is affected. Ordinal ranking of risks forms the basis of severity index:

$S = 1 + (r-1)/(n-1)$ where r = relative rank of risk and n = total number of risks identified (Smith et al., 2000). The most serious risks generate $s = 1$ while the least serious risks generate a value of 2. Merging the two dimensions enables identification of four categories of risks: common, yet severe risks: common, but less severe risks; uncommon yet severe risks; and uncommon, and less severe risks. By attaching the output to the georeferenced location of the communities that produce the data, that analysis generates spatially explicit risk maps (Stone, 2001; Smith et al., 2000) that potentially can integrate with hydrologic and land use modeling. Such integration acknowledges the multi-dimensionality of risk across ontological and epistemological worldviews since neither expert nor lay approaches to risk provides a complete understanding (Cohen, 2000; Lidskog, 2000; Rosa, 1998).

Regional Integration

We will work with Jennifer Olsen who is Co-PI at Michigan State University for the Land Use Change Impacts and Dynamics (LUCID) project in Kenya and Uganda in conjunction with the International Livestock Research Institute in Nairobi and the University of Nairobi. The LUCID project also addresses socioeconomic drivers of land use, gender dynamics, erosion and water quality (LUCID, 2004).

Schedule

Timeline: Literature review, Identify current development projects in the watershed: January 2005–June 2005.
Data collection in Kenya: July 2005–October 2005.
Lowlands (Kakamega and Webuye) – 3 weeks.
Upper Plateau Region (Eldoret and Kitale) – 3 weeks.
Highlands (Mt. Elgon and the Cherangany hills) – 3 weeks.

Data Analysis: October 2005–December 2005.
Final report to be submitted by 31 May 2006.

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Farmers Training in Tanzania

Economic/Risk Assessment and Social Analysis 3 (11.5ERA3)/Activity/Tanzania

Investigators

Kwamena Quagrainie	US Principal Investigator	University of Arkansas at Pine Bluff, Arkansas, US
Aloyce Kaliba	US Co-Principal Investigator	University of Arkansas at Pine Bluff, Arkansas, US
Kajitanus Osewe	HC Co-Principal Investigator	Fisheries and Aquaculture Development Division, Tanzania

Collaborators include partipants from the Department of Fisheries at Moi University, Kenya, Sokoine University of Agriculture, Mkindo Farmers Training Center, and Kingorila National Fish Center.

Objectives

1) Provide training on pond management, fish feed and fish health management.
2) Teach farmers principles and benefits of record keeping.
3) Teach farmers simple methods for assessing and evaluating costs and benefits.

Significance

This proposed training will involve 25 fish farmers from different participating villages in the Morogoro Region. In Tanzania, there is a lack of well-trained aquaculture extensionist at the village level. This is attributed to the organizational structure of the Central and Local governments. The ministry depends on farmers training workshop as a major mean of sustainable technology transfer rather than extension services. In addition, most aquacultural and on-farm research activities conducted by the Sokoine University of Agriculture (SUA) in collaboration with Kingorwila National Fish Center (KNFC) are in the Morogoro Region. It anticipated that future CRSP on-farm research activities would be conducted in the same region. The training is important for developing model-fish-farmers who will participate in future research activities and extending the knowledge to other fish farmers in the region. It is a training of trainers activity.

Anticipated Benefits

Farmers learn better from fellow farmers. The acquired knowledge will be used to improve productivity of their farms and extent the improved fish farming knowledge to other fish farmers. The training has a potential of accelerating the adoption process of improved technical innovations (through farmers to farmers knowledge transfer) and imparts self-confidence in fish farming among new farmers.

Research Design and Activities

A five working days training workshop will be conducted in collaboration with Mkindo Farmers Training Center (MFC). Three major topics will be covered: pond construction and management, fish health, fish nutrition, and fish economics. This will involve three persons from SUA, two person from KNFC, two persons from MFC and one person from UAPB. The number and gender of resource persons is to keep the workshop interesting. The workshop is scheduled to take place from September 18-24, 2005. The training activities will be based on training modules attached in Appendixes 2 to 5. The focus will be training of women and household members who manage the ponds. The names of participants are presented in Appendix 6.

Appendix 1

Schedule

September 18, 2005 (evening): Arrival of participants

September 19, 2005

Time	Event	Instructor/Trainer
07.30 – 08.30	Registration/breakfast	
08.30 – 10.00	Pond construction	Dr. Ngugi & Mr. Kajitanus (MSC) Senior Aquaculturist
10.00 – 10.30	Tea - Coffee	All
10.30 – 12.30	Pond Management	Dr. Ngugi & Ms. Yovita (MSc). Fisheries Biologist
12.30 – 02.00	Lunch	
02.00 – 07.00	Tour of Kingorwila Fish Farm	

September 20, 2005

Time	Event	Instructor/ Trainer
07.30 – 08.30	Registration/breakfast	
08.30 – 10.00	Common diseases of fish	Dr. Ngugi & Dr. Pereka PhD
10.00 – 10.30	Tea – Coffee	
10.30 – 12.30	Management of fish diseases	Dr. Ngugi & Ms. Regina (BSc) Fisheries Biologist
12.30 – 02.00	Lunch	
02.00 – 06.00	Tour of Vet. medicine laboratories	Dr. Pereka

September 21, 2005

Time	Event	Instructor/ Trainer
07.30 – 08.30	Registration/breakfast	
08.30 – 05.00	Field trip to two villages	
07.00 – 10.00	Welcome dinner	

September 22, 2005

Time	Event	Instructor/ Trainer
07.30 – 08.30	Registration/breakfast	
08.30 – 10.00	Tilapia culture: Production	Dr. Ngugi & Dr. Mnembuka, Ph.D Aquaculture Nutritionist
10.00 – 10.30	Tea – Coffee	
10.30 – 12.30	Tilapia culture: Fingerlings	Dr. Ngugi & Mr. Ntakwendera MSc Aquaculture Nutrition
12.30 – 02.00	Lunch	
02.00 – 07.00	Tour of SUA nutrition laboratory	

September 23, 2005

Time	Event	Instructor/ Trainer
07.30 – 08.30	Registration/breakfast	
08.30 – 10.00	Record Keeping	Dr. Senkondo PhD Agribusiness
10.00 – 10.30	Tea – Coffee	
10.30 – 12.30	Gross Margin analysis	Dr. Kaliba PhD Agricultural Economics
12.30 – 02.00	Lunch	
02.00 – 03.30	Pay back period	Dr. Kaliba & Dr. Senkondo
03.30 – 07.00	Exercises/discussion and feedback	

Appendix 2: Training MODULE: Record Keeping and Economic Analysis

Objective

The objective of this module is to teach farmers simple methods for assessing and evaluating costs and benefits and principles of record keeping. To impart the knowledge and concepts of assessing and evaluating costs and benefits of aquaculture activities, the most important thing is for the farmers to understand and appreciate the importance of record keeping as well as the status and importance of fish farming.

Introduction

Since the Tanzania's economy is based in rural area, currently, operation of aquaculture is dominated by smallholder farmers. However, aquaculture must compete with other rural livelihood options for land, water, labor and nutrients. This calls for deliberate efforts to evaluate the costs and benefits of aquaculture and its contribution to rural livelihood versus other undertakings. However, aquaculture is also faced with many problems and challenges including: Absence of a concrete policy for aquaculture;

Changes in economic policies; Lack of appropriate technologies; Lack of improved fingerlings; Poor transport infrastructure; Inadequate research; Lack of production records/statistics; Inadequate appropriate information management.

In order to be able to run any enterprise, it is important to have complete records of how the enterprise is run. In order to run a farming enterprise, the owner needs to have clear and complete records of all the operations carried out on the farm including records of all cash receipts and expenditures.

General Principles of Record Keeping
Good records should have the following characteristics:

- Should be accurate and used for the tasks that were intended
- Should be neat and easy to keep
- Should be complete and updated regularly
- One way of ensuring the above is to fill the records as soon as possible after the operation or transaction and check them regularly.

The main reason for keeping records is to assist in managing the enterprise specifically:

- To be able to measure the development of the enterprise
- To assist in making future decisions of the enterprise
- To have statistics for planning the enterprise/project

A satisfactory record keeping should fulfill the following:

- Easy to keep and understand
- Give all the information required
- Give information when needed

Record Keeping in Aquaculture
Considerations When Starting a Record System:
When designing a record system the following five things should be considered:

- The size of the business or number of fishponds;
- On a small business or with few fishponds, the farmer is also the record keeper. The volume handled for a year may be too small to employ a record keeper and the cost of record keeping system may be too high.

The Type of Business:
Most useful farm records depend on the type of farming. For example, a fish farmer may be interested more on the cost of the pond and revenue from the pond than any other thing.

The Training Required:
Training for improved record keeping is important. Such training should be provided by the extension services. For smallholder farmers records keeping should be done by simple and kept/managed by the farmers themselves. In addition, such records should prove useful for purposes of decision-making.

Time Required:
Since farmers time is mostly devoted to production activities (consider the fact that aquaculture is often done as a part time activity), less attention is placed on record keeping. To be interesting to smallholder farmers, record-keeping structure should be simple and should not be demanding in terms of farmer's time.

Use of Records*:*
Records can be used for the following purposes:
Evaluating the firm's financial success or progress over time.

> The farmer can easily determine whether he/she is operating at a profit or loss and if so how much profit or loss, consequently determine how much he/she can spend on himself/herself and his/her family.

To establish a factual basis for comparison with:
Performance with past years;
Goals that have been set;
Performance of other comparable operations; and
Other neighboring farms for which records are available.
They provide information for farm management decisions such as:
Financial control;
Evaluative performance;
Planning improvement in organization and operations; and
Expend the resource base.
Farm planning and budgeting:
Based on this kind of information, a farmer or farm manager can use his/her records to decide whether things are going according to plan and when modification is necessary, consistent with planning and control cycle.
To comply with tax reporting requirements:
Records may be kept to assist in tax planning and management.
Information arising from farm records can be very useful in acquisition of credit.

Components of Complete Farm Records:
- Inventory and depreciation schedule;
- Production and resource records (physical records); and
- Financial records.

Inventory and Depreciation: An inventory for a farm is a list of all farm assets and their value at a given time. Thus, inventory consists of physical count of farm items and valuation of these items.

Most often, the value of durable assets is determined after deducting their depreciation cost. Depreciation is an estimate of the amount by which the value of capital item falls in a given period because of
Wear and tear because of the equipment being used
Gradual deterioration of the equipment a function of time and age
Obsolescence, most equipment tends to go out of date over time as they become replaced by new and more efficient substitutes.

Production and Resource Records: There are a number of production and resource records that can be kept and efficiency measures calculated from them.

Financial Records: These are essentially accounts information, which can be categorized into financial accounts and management accounts. They include various statements derived from data on purchasing and selling of products.

Important Record Keeping for Aquaculture
Inventory Records: Equipment used in aquaculture and their values. These include hoes, jack, spades, fishing nets etc. In order to calculate their values over time we need to record their purchase price, when bought and the number of years that equipment is expected to last.. Table 1 can be used to keep such records.

Table 1: Equipment Used in Aquaculture

Item/equipment	Initial price (When bought) Tshs	Year when bought	Useful life of the equipment (years)
Hoe	5000	2000	10
Fishing net	10,000	2004	7

Records Kept under Fish Farming:

Under small-scale fish farming, the following can be considered. Do you own the land or rent? If you rent what is the rate per month/ year? This needs to be clear.
Data for fish farming can be recorded as shown in table 2 to 7.

Table 2: Data Sheet for Fishponds

Name of fish pond	Land own-ership Own =1 rent =2	If rented, Land rent Tshs	Length of the pond (m)	Width (m)	Depth (m)	Labour days	Expected useful life (Years)	Annual maintenance (Tshs)

Table 3: Other Information

Name of fish pond	Date of filling in water	Date of stocking fingerlings	Number of fingerlings	Species of fingerlings	Type of feeds	Amount of feeds

Table 4: Feeding

Name of fish pond	Type of feeds		Amount of feed used/day/week/ month		Price of feeds if bought		Fre-quency of feeding/ day/week	Labour hrs in feeding/ day
	Feed1	Feed 2	Feed 1	Feed 2		Price feed 1	Price feed 2	

Table 5: Harvesting

Name of fish pond	Date of harvest	Number of fish harvested	Average weight grams	Labour hours/ days

Table 6: Production, Consumption and Marketing

Name of fish pond	Number harvest-ed	Number Con-sumed at home	Number sold	Price (Tshs)	Number lost or free given	Labour hours/ days used

Table 7: Processing

Name of fish pond	Number harvested	Number dried/ smoked	Price of smoked fish	Number fried	Price fried fish (Tshs)	Labour hours/ days used frying/ smoking

Other Important Data to Be Recorded:

- Records of visitors (visitors' book);
- Comments raised by visitors;
- Records on unusual events happening in the pond; and
- Summary of income (from fish) and expenditure (from fish money).

Tools for Economic Analysis in Aquaculture: Fish farming is an investment activity whereby labor, land and capital resources are invested in raising fish. Substantial amount of labor is employed in excavating or constructing the fishponds. In evaluating the profitability of the investment, simple economic tools can be applied. Due to education level of participants, only two procedures will be introduced and this will include: gross margin analysis and payback period. The farmers will also be introduced on using records to develop simple line graphs and histograms to compare levels of inputs and outputs that cover different periods.

Gross Margin Analysis: Gross margin is the difference between gross value of output and the total variable costs used in the production process. As the starting point of financial analysis, this usually involves representative pond models. Based on patterns of representative ponds these models generate gross margins for aquaculture enterprises

Gross margin analysis is static, and does not take into consideration the time value of money. This is a deficiency when analyzing fishpond structures, which produce benefits over a number of years. However, it is a useful tool, which can assist in improving the overall management of the ponds as it addresses resource productivity in a given period.

Payback Period:
The pay back period i.e. number of years it will take to pay back the investment. This method can be able to rank projects, is simple to apply but does not consider revenue after the payback period. It is not a measure of profitability but of liquidity.

Appendix 3: Training MODULE: Pond Construction and Management

Objective
The training is designed to aid fish farmers to construct better ponds and manage the constructed ponds to maximize the harvestable fish. Not every aspect of private pond management can be covered. The focus will be on presenting basic information the farmers needs for developing and maintaining a good fishpond.

Introduction
If you are planning to construct a pond, contact your Village Aquaculture Extension Agent or people from the Ministry of the Natural Resources. These agents provide the technical engineering advice you need to properly design and construct a pond.

Fishponds should be of economic size. Smaller ponds seldom support a satisfactory fish population over many years. They usually require much more intensive fish management and may not justify the costs. Fishing ponds should have a drain line so the pond can be completely drained. The additional construction cost will result in shillings saved over the years. Drainage is important in fish management. Some pond owners believe that a deep pond provides better habitat (living space) for fish. This is seldom true. Most deep ponds don't contain enough oxygen for fish. It is only at higher temperatures when the circulate water is enough to supply oxygen to the deeper holes.

To ensure good water quality in your pond, do not allow livestock to wade in it. They trample the banks and muddy the water. If you need to water livestock at the pond, fence in a small area along the bank. Don't allow runoff from a barnyard or feedlot into the pond. Runoff from these sources adds excessive nutrients to the water and can produce obnoxious weed problems and cause fish kills. Avoid letting agricultural fertilizers and pesticides into the pond. Avoid plowing near the pond and reduce areas where soil erosion carries silt into the pond. Once you have a pond, it is important to know the exact acreage, maximum depth, average depth, and water volume. This information becomes useful in calculating the number of fish needed for stocking.

Chemical Features: The amount of oxygen dissolved in your pond's water is the most important chemical feature. Without oxygen, fish simply suffocate. If oxygen levels drop low, fish become stressed. Stress can then trigger secondary problems, such as poor growth, poor reproduction and easily diseases infections. Therefore, it is vitally important to maintain adequate amounts of oxygen in the water. For Tanzania, dissolved oxygen is not a major problem. However, when oxygen is less due to higher population, some fish may show signs of stress by gulping air at the water/air interface.

Biological Features: A pond is like the land around it. There is a limit to what it can produce. While a certain field can produce 10 bags of maize per ha another field can produce only 5 bags of maize per ha, a pond also has a limit to the amount of fish it can support. Just like the land, the upper limit or "carrying capacity" of a pond is influenced by fertility (nutrients available), climate and the type of crop being grown. The pond owner who understands the concept of carrying capacity will be able to manage and use the fish crop profitably.

Stocking Your Pond: After you have a properly constructed pond and a basic understanding of its features, it is time to stock your pond. You must consider what kinds of fish you want, how many and what size of fish you need to stock, when and how to stock, and potential stocking problems. Proper stocking can make a world of difference in fishing quality in years to come.

<u>What fish to stock?</u>
The stocking strategy you choose should be geared to the kind of fishing you want. If your chief interest is to raise an annual food crop, then tilapia would be best. If you simply want something in the pond to catch, just about any stocking combination will do. Well-oxygenated ponds may be able to support more fish and get a surplus for sale. However, this involved aeration and hence increase of investment.

<u>How and When to Stock Your Pond</u>
After you have decided what to stock, the next step is to locate a good source of fingerlings.
While catching adult fish from a nearby pond or creek and stocking them in your pond may be inexpensive and convenient, it can lead to several problems. Fish identification can be difficult, particularly of small fish. Stocking unidentified fish may certainly results to unpleasant surprises later on. These include stunted ness of the fish, difficulties in catching a proper number and equal size/age of fish, as well as increasing the chances of introducing unhealthy fish that may be diseased or injured. To invest a lot of money into the proper construction of your fishpond and follow it with poor stocking practices won't give you the return on your money that you expect. Kingorwila National Fish Farming Center is an important source for Tilapia fingerlings. These are supplied free of charge.

Stocking should not be delayed a bit once a pond has been fully filled with water. This is just to allow silt to settle at the pond's bottom. Moreover, it is during this period ponds' productivity is taking place depending on the amount and type of manure/fertilizer applied. Pond productivity is essential as the newly introduced baby fish has to get primary food already developed in the water. The time of year a pond is stocked is not important.

<u>Other Stocking Needs</u>
Properly managed, the initial stocking of tilapia is the only stocking you should ever have to make. However, regular re-stockings of new stocks of tilapia are important to maintain the "genetic vigor."

Managing Your Pond: Successful pond management requires more than just stocking fish. It is also important to maintain the proper environmental conditions, regular feeding, regular pond site visiting for fish enemies chasing, to monitor fish and growth and noticing diseases outbreak and clearing ponds' surrounding. This is the science of fishpond management, the solid basis for ensuring good fishing production.

How to Test Your Pond: The best way to tell how well your fish are doing is to practice regular fishing and weighing the fish and measuring the length. By so doing, you can check on how well your fish are growing, reproducing, fish healthy, the presence of the fish parasite or unwanted fish that have invaded the pond. You should fish your pond frequently, not only to learn how well your pond is doing, but also to harvest the older and larger fish as demanded by the market, personal preference or before they succumb to natural mortality. Keep a record of the fish you harvest from your pond. This will aid you in determining whether additional management is needed. There are several basic reasons why your pond may not produce the quality of fishing you want: some of these reasons are having wrong kinds of fish, inappropriate feeds and feeding, presence of fish predators and enemies, wrong number of fish or presence of non conducive ecological parameters within the water. Record the kind (species), weight, and length size of fish you catch. Then, periodically review your catch records. Ask yourself these questions:

1) Is the average size of fish declining?
2) Is the largest size of fish you catch getting smaller?
3) Do you catch fewer big fish per hour or more little fish per hour?
4) Are fish difficult to catch?
5) Are other non-stocked fish (species) showing up in the catch?

Answers to these questions can form a "common sense" approach to fish management in your pond.

Population Management:
A balanced pond fishery can be established with the initial stocking. This is usually the most difficult part of pond management. After the cost and effort of pond construction and fish stocking, the owner is understandably anxious to begin reaping the initial fishing benefits. Too often fishing leads to over-harvesting. When too many fish are removed, fish are free to overpopulate. The excessive number of tilapia fish quickly outstrips the supply of food and air. The result is having a stunted slow growing fish. To correct this situation, it is usually necessary to undertake periodical pond draining and start over with a correct number required to stock the pond or stocking only male Tilapia (monosex culture).

Soil Erosion Control: To reduce bank erosion caused by wave action, it is advised to plant grasses along the dikes. Allowing some aquatic vegetation, such as cattails and lilies, to grow in the dikes in not recommended as the harbor the presence of fish enemies. Livestock should be kept out of ponds. Muddy water caused by suspended soil particles can sometimes be corrected by digging ditches to allow silt settlement before the ponds' inlet. As the organic material decays, a weak acid is formed which causes clay particles to settle.

Pond Fertilization: Pond fertilization is based on the fact that the addition of nutrients to the water results in the increase of the pond productivity (i.e. production of plankton: microscopic plants and animals). This increases fish food, which results in increased fish production. However, fertilization can promote aquatic vegetation growth rather than plankton. Increases in aquatic vegetation can increase chances of fish kills as the vegetation decays and oxygen depletion. Plankton blooms can also occur due to over-fertilizing, causing the fish death as low oxygen supply. Once fertilization is started, it must become a permanent part of your management program or your pond's carrying capacity is reduced, often resulting in over-crowded, slow-growing fish.

Appendix 4: Training MODULE: Tilapia Culture

Objective

1) Training farmers on management and raising tilapia in ponds.

Introduction

Tilapia can be successfully raised in practically any farm pond. Ponds as small as 9m x 9m and 1m deep, can be used for tilapia culture. There are however, some problems involved with open pond culture.

1) Reproduction is uncontrolled. Spawning occurs about every 4 weeks as long as the temperature

is conducive. Some species of Tilapia are mouth brooders which means that the female shelters the young in her mouth after the eggs have hatched. During this time she is unable to eat and so grows at a much slower rate than the males; frequently reaching only half her potential size. Another problem arising from uncontrolled reproduction is large quantities of young which will not reach a harvestable size. They consume large quantities of food. They also lower water quality through their biomass excretes, that may contribute to stuntedness of the entire population and create sorting problems when fish of only a certain size are wanted for market. Therefore, in most cases, a means to control reproduction must be employed and this will be considered in a separate section.

2) Harvest can be difficult. Even in a pond designed for fish. Cultured tilapia can be difficult to seine efficiently. These fish tend to lie on their sides or burrow into the mud and under the net. This can be a severe problem in ponds filled with filamentous algae. Tilapias are also adapted at jumping over the net. A tall seine helps solve this problem. Partially draining the pond also makes the harvest easier. On a small scale, a lift net might prove a workable solution to the harvest problem.

Open Pond Stocking Rates: Open pond stocking rates for tilapia can vary considerably depending on the needs of the fish culturist and the production methods employed. For small or ponds that are only being supplementally fed with manures, grains or grain byproducts (i.e. semi-intensive fish culture), the fish should be stocked at a maximum rate of 15,000 per ha.

Open Pond Feeds and Feeding: At high densities, tilapia should be fed a regular fish ration with a protein value of 25-36% as they will rapidly deplete natural food sources. At lower densities, the fish ration can be fed or a variety of supplemental feeds may be used which can reduce costs of production. The fish should be fed about 3% of their body weight per day or all that they will consume in about 20 minutes. Tilapia grows much better when fed several times per day although growth may be adequate on a single daily feeding schedule.

Organic Fertilization: Organic manures have been used with success in many developing countries. Although these are used primarily to fertilize the water and increase natural production, the tilapia often search insect found in the manure or directly feed on these materials. Conversion factors are often poor but over all cost of production can be reduced significantly with the use of manures and crop wastes. Manures can be applied at a rate of 600 to 2000 kg/ ha initially, followed by repeat applications up to 50 kg per week per ha. Rate of application will vary with water temperature, dissolved oxygen levels, weather conditions and fish biomass. Manuring produces a bloom of phytoplankton, which the tilapias are able to feed on. Different species of tilapia vary in their ability to strain these microscopic plants.

Tilapia Fingerling Production: With many species of fish, breeding and fingerling production require some technical expertise or skill. This is not true of tilapia. Stock the brood fish in a ratio of 2 females to 1 male, at a density of 500-1000 fish per ha. Spawning begins when the water temperature exceeds above normal temperature. Depending on the environmental condition, most tilapia reaches sexual maturity at the age of 3 months. Depending on species, either males or females construct a circular nest 30-45 cm in diameter and 10-15 cm deep. Depending on size and other factors, female deposits 300-2000 eggs in the nest. The male then releases milt into the nest. Depending on species, male or female keeps on guarding the eggs, until the threat comes on. It is at this juncture when some species engulf the eggs or fry for defense.

The young fry are cared for about 8-10 days after hatching. During this time, they will venture out of the mouth cavity but remain near the mother, returning into her mouth if alarmed or in danger. This behavior insures a high fry survival rate even though relatively few eggs were produced. Females do not eat during the hatching period, that is why they tend to reach only half the size of males. Spawning takes place about every 4 weeks as long as the water temperature remains warm. A well-fertilized pond will provide the fry and fingerlings with an abundant food supply. However, growth will be more rapid if a high protein meal is fed supplementally. Six to eight weeks after hatching the fingerlings can be transferred to grow out ponds.

Controlling Reproduction in Open Ponds: Control of reproduction is probably the most important management problem in the culture of tilapia. If reproduction is left unchecked, the result will be a pond full of young and stunted fish, very few of which will reach a desired size at harvest. Many methods for control of tilapia reproduction have been used. Hybrid crosses of some species are often used because the resulting fish are all male and fast growing. Currently the most popular hybrid is the female *Oreochromis nilotica* with the male *Tilapia hornorum*. Sexing and crossing tilapias is not particularly difficult but does require some skill. It can also be difficult for the small fish farmer to find the right species to cross. SUA and KNFC are the key point to undertake this operation.

For this reasons, other means of reproductive control must be considered for the small-scale operation. Although in the future, it is likely that male hybrid crosses will be commonly available. Hand sexing of the fish for the selection of males is possible. The males are stocked in grow out ponds and the females are placed in brood ponds or discarded. This procedure has several problems associated with it. Sexing the fish accurately requires some skill and practice. A few females placed in the same pond with the the method useless.

The most practical method of reproductive control of tilapia for the small-scale farmer may be using of predatory fish. Catfish is probably the most popular and is effective in controlling fry and young tilapia fingerlings. Stocking rates will vary with the stocking rate of tilapia. In addition, tilapias have been used in polyculture with many different species of fish. In almost every case, the results have been beneficial. Total pond production has been increased without corresponding increases in the cost of production because the tilapia tends to clean up waste feed and algae that otherwise go uneaten by the primary species cultured.

Appendix 5: Training MODULE: fish disease and MANAGEMENT

Objective

1) Teach farms simple fish physiology for diseases diagnoses.
2) Teach farmers common diseases of fish and management.

Fish Disease Treatment

There are two broad strategies when administering fish disease treatments. Compounds such as copper sulfate, potassium permanganate, and formalin are added to the water to treat external parasite infections, external fungal infections and external bacterial infections (most commonly those diseases caused by the *Flavobacterium*). The compounds are added to the water to achieve a particular concentration. Most commonly the treatments are administered as prolonged treatments (hey are placed in the water and allowed to dissipate with time). The second strategy in disease treatment is the incorporation of an antibacterial compound into the feed. This is done to treat systemic bacterial infections.

The antibacterial is fed to the fish to provide a quantity of drug/fish weight per day for a specified number of days. The administration of medication via the feed necessitates that the fish are still actively feeding. Antibiotics are rarely administered as water baths in aquaculture, due to the large volume of water to be treated. Because of problems with resistance to the two available antibacterials, most fish health specialists consider it necessary to determine the antibiotic resistance pattern of particular bacterial isolate before the antibacterial treatment is applied.

Pathology—Common Parasites:
Slides will be used and farmers will identified common occurrences and a discussion will follow.

Pathology—Common Bacterial Diseases:
Slides will be used and farmers will identified common occurrences and a discussion will follow.

Pathology—Common Viral Diseases:
Slides will be used and farmers will identified common occurrences and a discussion will follow.

Pathology—Common Fungal Diseases:
Slides will be used and farmers will identify common occurrence and a discussion will follow.

Pathology—Common Food And Natural Vegitation Poisons:
Examples will be given to elaborate common food and natural vegetation poisons.

Appendix 6: Names of Participants

	Name	Gender	Village	District
1	Kibwana Tamimu	Male	Tangeni	Morogoro
2	Theodori Lolence	Male	Tangeni	Morogoro
3	Gervas Karoli	Male	Tangeni	Morogoro
4	Theresia Chande	Female	Mgeta	Morogoro
5	Junisia Mkude	Female	Mgeta	Morogoro
6	Kuluthum Omari	Female	Mkindo	Morogoro
7	Halima Juma	Female	Mkindo	Morogoro
8	Elizabet Mboma	Female	Kilosa	Kilosa
9	Melania Kachewa	Female	Isungo	Mahenge
10	Peter Nyamali	Male	Makanga	Mahenge
11	Athumani Kazumba	Male	Mkindo	Mvomero
12	Maria Pomoni	Female	Makanga	Mahenge
13	Hilary Chipeta	Male	Makanga	Mahenge
14	Mwinyimvua Selemani	Male	Mfumbwe	Mvomero
15	Ezekieli Changamno	Male	Luwale	Mvomero
16	Mama Nga'atigwa	Female	Luwale	Mvomero
17	Pankras Kampali	Male	Eponko	Mahenge
18	Rufina Gabriel	Female	Vidunda	Kilombero
19	Hija Mhondogwa	Male	Kalundwa	Morogoro
20	Inocent Magari	Male	Tangeni	Mvomero
21	Chambua	Male	Morogoro	Morogoro
22	Mstafa	Male	Mkindo	Mvomero
23	Kibwinamoto	Male	Mkindo	Mvomero
24	Swai	Male	Mkindo	Mvomero
25	Rutabingwa	Female	Mkindo	Mvomero

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

Applied Technology and Extension Methodology 1 (11.5ATE1)/Experiment/Bangladesh, Nepal, Thailand, and Vietnam

Investigators

Yang Yi	HC Principal Investigator	Asian Institute of Technology (AIT), Thailand
Md. Abdul Wahab	HC Principal Investigator	Bangladesh Agricultural Univeristy (BAU), Bangladesh
Madhav K. Shreastha	HC Principal Investigator	Institute of Agriculture and Animal (IAAS), Nepal
Nguyen Thanh Phuong	HC Principal Investigator	Can Tho University (CTU), Vietnam
P.T. Liem	HC Principal Investigator	Can Tho University (CTU), Vietnam
C. Kwei Lin	US Principal Investigator	University of Michigan
James S. Diana	US Principal Investigator	University of Michigan

Objectives

1) To adapt the integrated cage-cum-pond systems developed by Aquaculture CRSP to local conditions.
2) To determine appropriate stocking density of selected fish species in cages.
3) To assess growth and production of fishes in both cages and open ponds.
4) To assess the economic and environmental benefits of this integrated system.

Significance

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

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

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

Anticipated Benefits
This technology will provide small-scale rural farmers an opportunity to generate more income and improve their livelihood using their scare resources; and will benefit small-scale rural farmers and school students in Asian and other countries, where the integrated systems are practices.

Research Design
Location: Bangladesh, Nepal, Thailand and Vietnam

Methods:
Experiment B. On-Farm Trial (Activity)
Pond research in Bangladesh, Nepal, Thailand, northern and southern Vietnam. The on-farm trials will be conducted in collaboration with local government fisheries departments, NGOs and schools.

Pond Facility: Eighteen 200-400 m2 earthen ponds in each site.

Cage Facility: Various numbers of 4.0-m3 cages depending on pond size.

Culture Period: 150-200 days.

Test Species:
For Open Ponds: site specific based on local practices.
For Cages: stinging catfish in Bangladesh; sahar fish in Nepal; climbing perch in southern Vietnam, snakehead in northern Vietnam, hybrid catfish in Thailand.

Stocking Density: For open ponds, 1.0 fish/m2; stocking size: 8-10 g. For cages, the best density from the on-station trials, stocking size: 8-10 g. For cages in Thailand school ponds, hybrid catfish will be stocked at 250 fish/m3.

Nutrient Inputs: same as f.2.1.5.

Water Management: maintain at least 1.2 m depth.

Sampling Schedule: All water quality parameters mentioned before will be analyzed at beginning and end of the experiment. Fish size will be estimated at stocking and harvest.

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

Statistical Design, Null Hypothesis, Statistical Analysis: The design for the trial is a randomized complete block design. In each site except for Thailand, 3 areas will be selected with 6 ponds in each area, and 3 ponds will be used for the cage-cum-pond system, and other 3 ponds for control (normal fertilization practice). In Thailand, 9 rural schools will be selected with 2 ponds in each school, and one will be used for the cage-cum-pond system, and the other for control. The null hypothesis is that there will be no difference in fish production, economic return and water quality between the control and the cage-cum-pond system. The results of total fish production, water quality, and economic return will be analyzed for significant differences among treatments using ANOVA.

Regional Integration

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

Schedule

September 2004-May 2006. Report submission: May 2006 for on-farm trials.

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

Applied Technology and Extension Methodologies 2 (11.5ATE2)/Experiment/Thailand

Investigators

Amrit N. Bart	HC Principal Investigator	Asian Institute of Technology
Graham C. Mair	HC Principal Investigator	Asian Institute of Technology
James S. Diana	US Principal Investigator	University of Michigan

Objectives

1) Compare reproductive performance (age at sexual maturation, fecundity, spawning frequency, fertilization, hatch and larval survival) of three improved (GIFT, IDRC, Fishgen) and the Thai Chitralada strain of Nile tilapia.
2) Determine the relationship between fecundity and spawning frequency for the four strains of tilapia over time.
3) Compare the growth rates of the four strains in earthen ponds.

Significance

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

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

Currently there are at least three genetic lines in Asia in which significant improvement in growth rate has been achieved: 1) GIFT has undergone seven generations of combined selection (Eknath and Acosta, 1998), 2) IDRC strain has undergone 13 generations of within family selection (Bolivar and Newkirk, 2000), and 3) Fishgen-selected strain has undergone three generations of intensive selection on a stock of combined superior strains (Abucay and Mair, 2000). All these strains are now being bred at AIT. In addition the Thai Chitralada strain has developed a reputation throughout the region and beyond for its superior growth performance, despite not having been subject to any deliberate improvement efforts (Yakupitiyage, 1998). Although the selected lines are thought to have higher growth rates, there has been little independent verification of the gains, nor any comparison of the improved stocks with each other. In addition, there may have been a number of correlated responses to selection that could impact upon other traits such as reproduction and have not been subject to comparative evaluation. While the primary interest from tilapia producers is in the growth rates of these strains, hatchery managers need to know reproductive properties of these lines in order to manage them effectively. Some strains may have correlated traits, such as late maturation that are undesirable to seed producers and would severely limit their use, despite having attractive properties to growers. Given the interest in and demand for these improved breeds, their potential to boost yields and economic benefits in low input farming systems and the fact that they are already being widely disseminated, there is a need to quantify comparative reproductive capacity (as one of the most important of correlated traits). This study therefore, proposes to compare reproductive performances and growth of improved tilapia (e.g., GIFT, IDRC, UWS-CLSU) and compare them with Chitralada strain.

Anticipated Benefits

Successful completion of this study will:

1) Enhance understanding of the comparative reproductive potential of the improved strains as a trait correlated to growth and thus probably modified by selection;
2) Evaluate growth of the three improved varieties under at least two environmental conditions; and
3) Permit the formulation of recommendations for the appropriate use of one or more select groups based on reproductive performance and growth.

Research Design

Location: AIT, Thailand.

Methods: Experimental Fish: Selected GIFT (7th generation select) strain will be acquired from DoF of Thailand. The Fishgen-selected (third generation), IDRC (13th generation select) and the Thai Chitralada strains are held and will be bred at AIT. Brood fish will be held in hapas (5.0 m^3) in 335 m^2 surface area ponds. Fertilization and feeding regime will be followed based on previous studies.

Experiment 1. Reproductive Performance of Four Different Broodstock of Tilapia (GIFT, IDRC, Fishgen-selected, and Chitralada at AIT)

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

Stocking Density: Six-month old fish will be stocked at 24 per hapa (6m:18f). All females will be individually marked with PIT tags.

Fertilization and Feeding Regime: Ponds will be fertilized using 4N:1P and addition of urea or TSP if required. Additionally, broods will be fed 2% BW/d of pelleted commercial feed.

Sampling: Eggs from females will be collected at 7 day intervals, their stage of development determined and then incubated using standard tray systems (Little 1989) in the AIT hatchery. The following data will be collected over a 22-month period that should encompass the period of optimal production by the broodstock:

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

In addition visual assessment of general conditions of the broodfish, eggs, sperm, embryos and larvae will be made to compare with observed numbers. These offspring will be held separately and used in the conduct of Experiment 2.

Experiment 2. Comparisons of Growth Rates and Sexual Maturation Between the Four Strains of Tilapia in Two Environments (Semi-Intensive and Intensive)

Comparisons will be done with mixed-sex fish with separate stocking (12 x 200 m^2 ponds) and in mixed sex and monosex stocking (1 x 200 m^2 pond each) under communal stocking. All fish will be stocked at the same age, at a mean weight of 5g, with communally stocked fish marked with a combination of coded wire tag and fin clipping.

Pond Facility: 14 earthen ponds of 200 m^2 surface area will be used for each environment. We will maintain water depth of 1.0 m.

Culture Period: 150 days

Base Stocking Densities: 2.7 fish per m^2 in open ponds.

Nutrient Input: Fertilize ponds at a rate of 4N:1P for the extensive system. Feed pelleted feeds at 5%, 3.5% and 2.5% BW/d during the first, second and third months for the full feeding system

Water Quality: standard CRSP protocol. Fish growth and sexual maturity will be sampled every three weeks. To assess the rate of gonad development and time of sexual maturation, 10-20 males and 10-20 females will be removed from the pond and sacrificed every 21 days. Initial stocking rates will be increased to compensate for reduction in density caused by this sampling.

Experimental Design, Null Hypothesis and Statistical Analyses:
Experiment 1. Variables measured include spawning, spawning frequency, fecundity, hatching rate and survival rate of fry.

Null Hypothesis: There is no difference between four groups of tilapia in relation to reproductive performance over time.

Statistical Analyses: Significant difference between means will be analyzed using ANOVA. If treatment differences are observed, Least Significant Difference tests will be used at α=0.05 level of significance to determine which groups differ from each other.

Experiment 2. Three replicates of four treatments (strains of tilapia) in which growth means will be compared between treatments and in two different environmental conditions (extensive and full feeding). Also, two sex ratio "environments (mixed-sex and monosex – communal stocking only) will be tested for significant differences.

Null Hypotheses:

1) There are no differences in growth rates and sexual maturation between the four strains of fish tested in the two different environments.
2) There is no difference in the relative growth of the strain under communal and separate stocking.
3) There is no difference in the relative growth of the strain in mixed sex and monosex environments.
4) An overall null hypothesis: there is no genotype environment interaction in the relative growth of the four strains.

Statistical Analyses: Significant difference between four different treatment means and two sites will be analyzed using ANOVA and regression. If treatment differences are observed, pair comparisons will be made using LSD (α=0.05).

Impact Indicators

A best performing strain may be identified both in terms of growth and reproduction. Results of this have implications for previous CRSP studies on growth performance of tilapia and will be used to compare, contrast, and assess relative improved performance. Dissemination of information in scientific papers and popular articles will inform farmers of the respective properties of the strain and promote appropriate adoption of the improved breeds by different sectors of the tilapia industries in the region.

Regional Indicators

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

Schedule

Experiment 2: September 2004 to May 2006. Report submission: July 2005.

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Aquaculture Training for Kenyan Extension Workers, Fish Farmers, and University Students

Applied Technology and Extension Methodologies 3 (11.5ATE3)/Activity/Kenya

Investigators

Charles C. Ngugi	HC Principal Investigator	Department of Fisheries, Moi University
Bethuel Omolo	HC Principal Investigator	Fisheries Department, Government of Kenya
Chris Langdon	US Principal Investigator	Department of Fisheries and Wildlife, Oregon State University
James Bowman	US Principal Investigator	Department of Fisheries and Wildlife, Oregon State University

Objectives

1) Train up to 34 extension workers and 6 advanced farmers in hatchery management techniques at government or university farms.
2) Provide on-farm training for up to 12 farmers in simple techniques for spawning, hatching, and rearing catfish juveniles in ponds.
3) Provide support for four undergraduate university students training in aquaculture.
4) Provide support for two university students for Masters-level (MSc) training in aquaculture.

Significance

Efforts to grow fish in ponds in Kenya began in early 1920. However, rural fish farming dates back to 1940s when farmers in Central and Western Kenya started to construct fish ponds to culture Nile tilapia. In spite of several decades of fish culture, Kenya's aquaculture has not come far and remains a young industry, practiced mainly on a small scale using Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) and producing only about 1,000 metric tons annually (Kenya Fisheries Department, 2000).

The African catfish, *Clarias gariepinus* is probably the most widely distributed fish in Africa (Skelton, 1993). It can endure very harsh conditions by using its accessory air breathing organ, is highly omnivorous, and grows quickly. The fish has high potential for aquaculture in this region. Research into the culture potential and the artificial propagation of catfish is reported to have begun in the 1970's. (DeKimpe and Micha, 1974; Hogendoorn, 1984).

Clarias gariepinus is endemic to the Lake Victoria region. It is popular with the communities living around the lake and a lot consider its taste as excellent. *Clarias* are farmed in polyculture with Nile tilapia to control unwanted reproduction. They are also grown in monoculture as food fish. More recently, *Clarias* culture has gained in importance as a way of producing bait fish for the Lake Victoria Nile perch industry. The traditional supply of bait fish is wild-caught catfish fingerlings from Lake Victoria itself, but this supply is intermittent and seems to be related to the extent of water hyacinth rafts drifting in near shore, with *Clarias* being numerous under the water hyacinth. Fishers sometimes use small-mesh beach seines and mosquito nets to catch fingerlings for bait, but beach seining is highly destructive of the spawning habitats of native cichlids and is illegal (Abila, 2000). Fishing with mosquito nets has also recently been banned by the government. Fishers find themselves in a difficult situation because they need the bait at an affordable price to be able to continue fishing.

Like many other indigenous fish species tried in aquaculture, lack of adequate seeds is a drawback to production of catfish. Spawning of *Clarias* is not a major problem, but sufficient quantities of fingerlings cannot be produced due to very low survival to fingerling size. Spawning success as reported by Karen Veverica (Personal Communication) was quite good, but survival to fingerling size was variable, ranging from 1 to 50% in ponds, with a survival rate of 25% from egg to 5-gram fingerling considered good. Further work to increase survival to the fingerling stage therefore needs to be done.

Some improvements in *Clarias* survival were realized through experiments conducted at Moi University (MU) under CRSP sponsorship in 2002 and 2003. Those experiments included studies on suitable stocking densities, best first feeds, and the benefits of providing shading and cover for fry reared in hapas in outdoors tanks. The feeding experiment demonstrated that pond-cultured rotifers can successfully and economically be used as first feeds for *Clarias* fry, and the aquarium stocking density study suggested that the best survival and growth can be achieved when larvae are stocked at about 10 per litre. In the pond/hapa experiments, it was shown that lower stocking densities—around 100 larvae/m^2—gave better survival and growth than higher stocking densities (200 and 400 fry/m^2). Finally, provision of 100% shading over hapas placed in ponds resulted in better growth and survival of fry than did provision of less shade (60, 30, and 0% coverage) (Ngugi et al., 2004a, 2004c).

The gains made through those experiments now need to be shared with extension workers and with advanced farmers anxious to improve *Clarias* fry production. Previous short courses supported by the CRSP focused on basic aquaculture knowledge such as evaluating potential pond sites, constructing and managing ponds, and the economics of running fish farming enterprises (Ngugi et al., 2004b; Ngugi et al., in press). These new proposed courses will focus specifically on the production of *Clarias gariepinus* fingerlings, and will target extension workers and farmers who need this specific type of training. Training of selected Fisheries Assistants (FAs) of the Kenya Fisheries Department (FD) (referred to as extension workers), selected producers, and other workers intimately connected with *Clarias* production efforts will be conducted to achieve this end. In addition, support will be provided for two graduate students who will pursue masters degrees while conducting research on additional factors affecting the growth and survival of *Clarias gariepinus* fry (see related proposal in this packet entitled "Studies on Strategies for Increasing the Growth and Survival of African Catfish (*Clarias gariepinus*) Juveniles Reared for Stocking or for Use as Bait").

Quantified Anticipated Benefits

Extension workers and fish farmers who have been trained in the latest, best techniques will be able to apply that knowledge to increasing the production of *Clarias* fingerlings on both government and private farms. An increased supply of *Clarias* fingerlings will provide Lake Victoria Nile perch fishers with a reliable source of bait. Fishing pressure on immature *Clarias* in Lake Victoria will be reduced. Reduction in beach seining will reduce habitat destruction on native fishes in Lake Victoria. Net income to fishermen may increase if baitfish is more available and if costs are kept down through competition among bait producers. 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. As with the *Clarias* fingerling production research to be conducted under this project, increases in fish production realized through all these avenues will contribute to human health and welfare in the region.

On the outreach/educational side, MU and FD course instructors will gain valuable experience in the practical aspects of *Clarias* spawning and rearing as well as in methods of teaching these techniques to farmers and extension agents. The aquaculture handbook prepared as part of an earlier CRSP/Kenya project can be modified or improved following these training courses, both with respect to its organization and the technical information it provides.

Finally, collaboration on this project will strengthen both of the primary institutions involved—the MU Fisheries Department and the Kenya Fisheries Department—as well as further strengthen the linkage between them. Graduate training provided for FD personnel will further build on the strengths of that Department.

Research Design

Location of Work: Short courses for extension workers will be offered at either the Moi University Fish

Farm (Chepkoilel Campus) in Eldoret, Kenya, or at Sagana Fish Farm, Sagana, Kenya. Farmer training will be conducted at the farms of key collaborating farmers in Kenya. Undergraduates attending Moi University will carry out their senior project work at either the Moi University Fish Farm or Sagana Fish Farm. Master's degree (MSc) training will be conducted at Moi University, Eldoret, Kenya.

Methods:

Pond and Hatchery Facilities: At Chepkoilel, 12 ponds measuring 10 x 10 (100 m^2) and /or 12 hapas, and 24 30-L indoor aquaria. At Sagana, 12 ponds measuring 10 x 15 m (150 m^2). Pond culture may be preceded by nursing of fry in the indoor hatchery at either location.

Short Courses for Extension Workers and Advanced Farmers: Two two-week short courses will be offered to selected FD Fisheries Assistants (extension workers), KMFRI research officers, advanced fish farmers, or other individuals as deemed appropriate. Twenty individuals will be trained in each session. These courses will focus on the whole fingerling production process, from maintenance of *Clarias* broodstock through brooder selection, spawning, incubation, hatching, and rearing of fry through the first 21-28 days after hatching. In the two or three weeks prior to each session, trainers will produce several batches of fry at staged intervals to make fish of all relevant ages available to the trainees, so that the entire production process can be covered within the two-week time frame of the courses. Farmers who participate in this training will likely be the ones who subsequently host on-farm farmer training sessions as described under "Farmer training" below. Trainers for these courses will be selected from among MU and FD personnel who have worked on *Clarias* production and research during the past few years. A tentative outline for these courses is attached as Appendix A.

Farmer Training: Farmer training will be conducted at the farms of two selected leading farmers in western Kenya. Insofar as possible, these leading farmers will have participated in the two-week training sessions described above under "Short courses for extension workers and advanced farmers" prior to hosting other farmers at their own farms. Groups of up to six promising farmers from the host-farmers' areas will be invited to come to their farms for periods of up to one week. Two of these one-week sessions will be conducted during 2005 and early 2006. The training will consist of hands-on spawning/hatching/rearing work conducted by the farmers themselves under the guidance of the host farmer and one or more experienced technicians from either MU or the FD. Trainers will teach the best available techniques from published research results, local experience, and recent CRSP-sponsored experiments that are practicable under local conditions. FD or MU technicians who assist the host farmers will be selected from among those with prior *Clarias* hatchery experience, for example individuals who have been working with *Clarias* at either Sagana Fish Farm or the MU Fish Farm or recent MSc graduates who worked on CRSP *Clarias* production experiments in 2002 and 2003. A tentative outline for these on-farm training sessions is attached as Appendix B.

Undergraduate Stipend Support: Short-term stipends will be provided to support senior project work for four selected undergraduate students studying in their final year at Moi University. Research topics will cover areas such as catfish fecundity, condition factor, water quality management, disease control, and other topics relevant to the growth and survival of catfish juveniles. Moi University faculty members will supervise these students.

Masters Degree Training: Two officers selected by the FD will be provided full support (full tuition and living stipends) for up to two years while they conduct research to improve *Clarias* seed production techniques and work towards earning masters degrees (MSc). One will be selected from the Kenya Fisheries Department while the second will be selected from among graduate students registered in the Moi University Department of Fisheries. They will conduct their work under the direct supervision of Charles Ngugi (Head, Department of Fisheries, Moi University) and James Bowman (Department of Fisheries and Wildlife, Oregon State University), with further advisory help from Bethuel Omolo (Head of Station, Sagana Fish Farm, Kenya Fisheries Department). These students should begin their work as soon as possible, and not later than 1 January 2005. Coursework should begin in January 2005 and research should be conducted from June – December 2005. Coursework should begin in January 2005 and research should be conducted from August 2005–February 2006. Students will submit their theses by April 2006.

Regional Integration

These training activities directly address Objective 2 in the CRSP Regional Plan for Africa, which is, "To assist in the development and conduct of aquaculture training courses and programs, with emphasis on pond operation and management." They also address the overall goals of strengthening research and extension institutions in the region and encouraging technology transfer among research and extension institutions in the region. Furthermore, they address a key aspect of overall CRSP objectives for the current grant, which is "the development of culture systems for local and native species" (New Aquaculture Systems/New Species Theme).

Schedule

Short Courses for Extension Workers and Advanced Farmers:

These courses are to be conducted during the summer (May – September) of 2005.

Farmer Training:

These courses are to be conducted during the fall (September - November) of 2005.

Masters Degree Training:

- Students will be selected in September 2004.
- Course work will begin as soon as possible after selection, but not later than 1 January 2005.
- Students will begin writing their research proposals as soon as they begin their coursework.
- Pond and/or hatchery research will be conducted between August 2005 and February 2006.
- Theses will be ready for presentation to committees by 31 April 2006.
- The status of all activities will be regularly submitted in CRSP quarterly reports.
- A final report on these training activities will be submitted to the CRSP by 30 May 2006.

Tentative Two-Week Extension Workers' Training Program in Hatchery Management Techniques for Propagation of African Catfish

Duration: Two weeks.

Target Group: The course is designed for Fisheries Assistants and advanced fish farmers.

Requirements: Participants must have a minimum of primary school education.

Major Topics:

1) Simple techniques for the management of catfish juveniles
   Water quality monitoring
   Pond fertilization
   Stocking density in ponds, cropping and harvesting
   Sanitation practices

2) Seed and larva l production
   Methods of Catfish seed production
   Hatchery production techniques
   Broodstock collection
   Spawning techniques
   Fertilization
   Incubation and hatching
   Causes of egg mortality and their treatment
   Larval rearing
   Mass fry production of catfish and other fish.
   Transportation of fish seed

3) Fish Nutrition and feed formulation
Nutritional requirements of fish
Protein, Dietary energy, Vitamins, Minerals, Essential lipids
Feeding regimes, Feed formulation and processing

4) Fish health management
Introduction to stress and the disease process
External signs of infection in fish
Types of fish diseases
Methods of disease control, Preventive husbandry
Methods of disinfection, Principles of quarantine
Mass drug therapy, Immunization with vaccines

Tentative Schedule:

Day	**Activities**
Week One	*Registration, stationery supply & training materials*
Day 1	Opening remarks: Principal Chepkoilel Campus and Director of Fisheries Self introduction of all training staff and trainees Explanation of training materials that will be used. Objectives of training program. Administrative questions Tour of training site fish farm and the hatchery.
Day 2	Discussion on biology of catfish Seining for *Clarias,* handling brooders, sexing and pituitary injection.
Day 3	Removal of pituitary from *Clarias* for Hypophysation, other methods of breeding catfish Stripping, fertilization & incubation of *Clarias* eggs Soil Qualities and Liming
Day 4	Preparation of ponds for fry stocking Fertilization of Ponds and Calculation of Fertilizer and Manure Application Rates
Day 5	Observation of incubated *Clarias* eggs for hatching Fieldwork estimation of *Clarias* and stocking fry in hapas Introduction to Water Quality management Monitoring catfish growth performance
Day 6	Hatchery Nursing of catfish fry Monitoring catfish growth performance
Day 7	Rest day
Week Two	*Monitoring catfish growth performance*
Day 8	Fish Feed and Nutrition (Juveniles)
Day 9	Monitoring catfish growth performance
Day 10	Monitoring catfish growth performance
Day 11	Field visiting to Fish Farmers' Farms
Day 12	Discussion on Extension Services
Day 13	Course assessment
Day 14	Presentation of Certificates and Closing

Tentative One-Week Catfish Farmers' Training Program

Major Topics:
- Seed and Larval Management;
- Spawning process both in Nature and in the hatchery;
- Basic equipment used;

- Selection of Spawners;
- Collection of pituitary;
- Fertilization and incubation of eggs;
- Larval development Nursing fry growth and survival of fry;
- Fish health management; and
- What have we learned so far.

Schedule:

Day	**Activities**
Week One	*Registration, stationery supply & training materials*
Day 1	Opening remarks Self introduction of all training staff and trainees Explanation of training materials that will be used. Objectives of training program. Administrative questions Tour of farmers site
Day 2	Discussion on biology of catfish Seining for *Clarias*, handling brooders, sexing and pituitary injection. Simple techniques in propagation of catfish
Day 3	Further discussion on breeding catfish fertilization & incubation of *Clarias* eggs Fieldwork estimation of *Clarias* fry spawned
Day 4	Soil Qualities and Liming, Preparation of ponds for fry stocking Fertilization of Ponds and Calculation of Fertilizer and Manure Application Rates
Day 5	Monitoring catfish growth and survival Discussion on constraints in catfish fry production
Day 6	How to nurse catfish fry Discussion
Day 7	Presentation of Certificates and Closing

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

Applied Technology and Extension Methodologies 4 (11.5ATE4)/Activity/Mexico

Investigators

Carl B. Schreck	US Principal Investigator	Oregon State University
Guillermo R. Giannico	US Co-Principal Investigator	Oregon State University
Wilfrido M. Contreras-Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Ulises Hernández-Vidal	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Bernardita Campos-Campos	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico

Objectives

1) Conduct three workshops on safe handling of steroids and masculinization techniques in Guatemala, Costa Rica, and Honduras.
2) Distribute printed and electronic material for safe handling of steroids and masculinization techniques.

Significance

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

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

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

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

Anticipated Benefits

From our previous work, we have produced a manual on masculinization of tilapia and safe handling of steroids and designed posters to train farmers and extension agents. However, we were not able to finish a web page to disseminate information on clean technologies and elimination of MT, nor to translate the manual to English. Therefore, we intend to finish these tasks during in the following months. Workshops conducted in Central America will educate extension agents, technicians, and farmers on safe and effective sex inversion techniques. These personnel can then train additional growers. A manual (both hard copy and web-based) in English and Spanish, that has been produced at UJAT, will be disseminated to high school and university students as well as the personnel listed above.

Activity Plan

Activity 1: Three workshops will be conducted in Antigua, Guatemala; Tegucigalpa, Honduras; and San Jose, Costa Rica concerning sex inversion techniques, safe handling of steroids, and treatment methods for effluents. Officials and Universities will be contacted to create a network of farmers who are interested in participating. If necessary, we will conduct workshops in outlying areas. Non Governmental Organizations involved with farmers will be contacted to help create the workshop network. They will play an important role for local organization.

Schedule

Workshop organization and implementation, November 2004–June 2006; Final technical report, 30 June 2006.

Regional Integration

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

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Establishment of the Center for Aquaculture Technology Transfer

Applied Technology and Extension Methodologies 5 (11.5ATE5)/Activity/Mexico

Investigators

Eunice Perez-Sanchez	HC Principal Investigator	Universidad Juarez Autonoma de Tabasco
Margarita Cervantes Trujano	HC Co-Principal Investigator	Instituto Tecnologico del Mar, Veracruz
Dale Baker	US Principal Investigator	NY SeaGrant
John Jacob	US Co-Principal Investigator	Texas SeaGrant
Ralph Rayburn	US Co-Principal Investigator	Texas SeaGrant
Michael Timmons	US Co-Principal Investigator	Cornell University
David Belcher	US Co-Principal Investigator	Cornell University
Martin Schreibman	US Co-Principal Investigator	Brooklyn College
Barry Costa-Pierce	US Co-Principal Investigator	URI/SeaGrant
Ruperto Chapparro	US Co-Principal Investigator	Puerto Rico Sea Grant
Kevin Fitzsimmons	US Co-Principal Investigator	University of Arizona
Martin Hevia	HC Co-Principal Investigator	La Fundacion Chile

Objectives

1) Recruit key Aquaculture research and extension personnel within the Mexican university and institution system.
2) Conduct an organizational meeting of the Center for Aquaculture Technology Transfer (CATT) to determine center goals, director, and group structure.
3) Initiate the CATT program.
4) Establish ties with US-based researchers and SeaGrant Institutions to create basis for on-going collaborative research and extension activities.

Significance

Nearly 40% of the seafood needed to feed the world's growing population (estimated demand of 150 million tons by 2010) is expected to come from aquaculture expansion (FAO, 2004). Aquaculture growth over the past 30 years has encountered several economic, environmental, and biological constraints such as price fluctuations, mangrove destruction and viral and bacterial challenges that have led to the need for sustainable aquaculture planning and information exchange to reduce these risk factors and mitigate the pressure for more fish without concern for the environment. The USAID has expressed a need for US cooperation in sustainable aquaculture development around the world and recently indicated that,

> "The sustainable development and management of aquaculture and fisheries systems can only occur if these activities are well planned and integrated into the natural and social resource, ecosystems, and farming systems of the large global context of which they are a part." (USAID)

Dissemination of technical information as part of extension outreach to producers is thus a critical aspect of sustainable aquaculture development. Establishing an effective large-scale extension service for a developing and multi-disciplined industry is a considerable task when there are many different economic, environmental, and technical considerations needed for project success for a variety of stakeholders. Relevant research in sustainable aquaculture is being conducted within Mexico and the US today such as the ACRSP projects to study the sustainability of tilapia-shrimp polyculture and the training of farmers to safely handle steroids and masculinization techniques. However improved communication and extension between researchers, educators, extension agents and aquaculture producers are still needed for increased implementation and adaptation of sustainable technology.

Initial efforts to expand and strengthen extension outreach services in Mexico have recently been initiated. A consortium of US and Mexican universities along the Gulf of Mexico met in the summer of 2004 to discuss some of the research and extension challenges facing the industry. Out of this meeting, several Mexican university researcher/extension personnel were identified as extension collaborators and a listserv and website were created to support their effort. In addition to this effort, a considerable portion of current CRSP/USAID research projects in Mexico, such as the Santa Maria Bay Management Project and collaborative tilapia research in Tabasco on training local farmers in safe handling of steroids and masculinization techniques, have included extension workshops and outreach as part of their plans (CRSP Eleventh Work Plan).

This project aims to continue this effort and expand it by establishing a center for aquaculture technology transfer that is *narrowly focused* in its scope. We will call this center the Center for Aquaculture Technology Transfer (CATT) and will be patterned after the US SeaGrant Program model. The US SeaGrant Program, which is administered through the National Oceanic and Atmospheric Administration (NOAA), is a nationwide network of 30 university-based programs that work with coastal communities (NOAA SeaGrant). The National Sea Grant College Program engages this network of the nation's top universities in conducting scientific research, education, training, and extension projects designed to foster science-based decisions about the use and conservation of aquatic resources. The US SeaGrant program is focused on eleven themes and three national priority areas. The themes of interest and study under SeaGrant include aquaculture, biotechnology, coastal communities and economies, ecosystems and habitats, fisheries, and aquatic invasive species to name a few.

Our proposed project intends to take the same approach to setting up a SeaGrant-type program in Mexico; however it will initially be focused on one central theme: sustainable aquaculture development. Concentration on a single theme, though broad in scope, will streamline the center's effort and increase its effectiveness. As the program develops, its extension services can be expanded to include additional themes that are important to the country and region.

The CATT will be a virtual network of aquaculture research and extension universities and institutions that are united through a single mission, a central website, and a director. The overall mission of the CATT will be to enhance the implementation and adaptation of sustainable aquaculture technology and information from research, economic, and regulatory sources to aquaculture production stakeholders. The CATT will also serve as a uniting entity among members to steer/guide research priorities and possibly even disseminate research funding. The way in which the CATT carries out its mission including its organizational structure, its director, and its priority interest areas will be determined by its membership. This project will support the creation and operation of the CATT by hosting an organizational meeting, providing SeaGrant coordination and extension guidance, providing salary support to the CATT director, and providing infrastructure support (e.g., internet/web service, telephone, and office materials).

The US cooperators that will assist is this project include Dale Baker of Cornell NY SeaGrant, Michael Timmons of Cornell University, David Belcher of Cornell University, Martin Schreibman of Brooklyn College, John Jacob of Texas SeaGrant, Barry Costa-Pierce of URI/SeaGrant, Kevin Fitzsimmons of University of Arizona, Ralph Rayburn of Texas SeaGrant and Ruperto Chapparro of Puerto Rico Sea Grant.

The HC principal investigator will be Eunice Perez-Sanchez of the Universidad Juarez Autonoma de Tabasco. Margarita Cervantes Trujano of the Instituto Tecnologico del Mar located in Boca del Rio, Veracruz will serve as HC Co-PI. Collectively, Cervantes Trujano and Perez-Sanchez have extensive university contacts throughout Mexico and have expressed eagerness in promoting sustainable aquaculture extension. Additionally, Perez-Sanchez was a Co-Principal Investigator on a CRSP project (Training Local Farmers Safe Masculinization Techniques), which has involved the training of extension agents, farmers, and students through a series of workshops.

A table indicating the project membership, expertise and anticipated role is presented below:

Project Team

Team Member (Affiliation)	Expertise	Role in CRSP Project
Dale Baker (Director NY Sea Grant Extension)	NY Sea Grant extension methodologies, project research and management	Lead PI, responsible for all reports and budgets
Michael Timmons (Professor, BEE, Cornell University)	Leading authority and researcher in recirculating aquaculture systems; engineering entrepreneurship; ag. extension	Lead role in aquaculture workshop development and recirculating aquaculture system design
David Belcher (Extension Associate, BEE, Cornell University)	Commercial aquaculture production management; environmental engineering; agriculture extension	Project management and participation in all project phases, lead coordination with HC PI to ensure all activities are completed on time
Martin Schriebman (Professor, Brooklyn College)	Fish biology, fish endocrinology, estuary and fisheries management in economic development	Presenter at aquaculture workshop; collaborator in establishing CATT network and recruiting US counterparts
Ralph Rayburn Associate Director Texas Sea Grant College Program and Sea Grant Extension Program Leader	Policy and regulations associated with the coastal and marine environment, program management, commercial trade association coordination	Collaborator in setting up CATT model (organizational meeting) and in recruiting US counterparts into support network
John Jacob (Director, Texas Coastal Watershed Program, Texas Sea Grant)	Watershed management, sustainable urban development, wetlands, natural resource mapping and assessment	Collaborator in both CATT set up (organizational meeting and web site) and presenter in Aquaculture workshop
Barry Costa Pierce (University of Rhode Island Sea Grant)	Sustainable aquaculture, tilapia genetics, international aquaculture development	Collaborator in CATT set up, Presenter at Aquaculture Workshop.
Kevin Fitzsimmons (Professor, University of Arizona)	Aquaculture production; tilapia breeding and hatchery techniques; international extension	Collaborator in CATT set up, Presenter at Aquaculture Workshop (tech transfer from other ACRSP project)
Ruperto Chapparro (University of Puerto Rico Sea-Grant)	Sea Grant Extension (in both English and Spanish)	Collaborator in CATT set up (organizational meeting) and recruiting US counterparts
Martin Hevia (Director of Scientific Programs, Fundacion Chile)	Aquaculture production; program management	Presenter and assistant organizer of Aquaculture Workshop
Eunice Perez Sanchez (Professor, Universidad Juarez Autonoma de Tabasco)	Sustainable aquaculture; fisheries management; coastal resource management; economic development	Host Country PI responsible for coordination, management, and budgeting of all in-country activities
Margarita Cervantes Trujano (Professor, Instituto Technologico del Mar)	Aquaculture systems, shellfish and finfish production	Host Country co-PI to recruit in-country network members; participate in all meetings and workshops

Anticipated Benefits

Establishment of the Center for Aquaculture Technology Transfer (CATT) is the next step towards a Mexican extension outreach program focused solely on sustainable aquaculture development. The CATT network that is established from this work will be based upon the SeaGrant model and built upon recent extension activities taking place in discreet locations in Mexico and will aid in keeping researchers, extension personnel, and farmers throughout Mexico current on research and production technology advancements. Implementation and adaptation of the CATT program will also continue to develop the channels of communication between different resources in the US and Mexico for aquaculture development and will set the stage for future cooperative research and extension activities between nations.

Activity Plan

Activity 1. Identify and Recruit Potential Members of the CATT Network and Determine Status/Needs for Sustainable Aquaculture Extension in Mexico

Introductory correspondence will be initiated between HC PI's and all known aquaculture-related researchers, industry, and government institution personnel that would likely have an interest or stake in the CATT center. Although the bulk of this group will be university based, contacts within the government and industry groups are also viewed as key contributors towards the development of the CATT. Experience and contacts made through previous CRSP projects such as the Santa Maria Bay Project, the polyculture of tilapia and shrimp, and the training of local farmers in safe handling of steroids and masculinization will form a strong starting network link. To achieve geographical and sustainable aquaculture topic coverage, the project has an initial recruitment goal of 12 CATT members. Initial correspondence will be to identify and recruit potential CATT extension network members, determine initial thoughts and ideas regarding the current status and needs for sustainable aquaculture extension in Mexico, ideas on how the CATT can serve to meet these needs, and identify representatives from each Mexican State to attend the organizational meeting. Recommendations and information from initial correspondence will be compiled and analyzed by HC and US Co-Principal Investigators. US co-principal investigators will provide recommendations on establishing the CATT based upon SeaGrant and extension experience. Processed information and recommendations regarding the CATT will then be disseminated back to interested stakeholders who will form the overall CATT extension network.

Cervantes Trujano and Perez-Sanchez will be the primary Co-Principal Investigators responsible for completing this task. All of the U.S Co-Principal Investigators will assist in this initial task to ensure that the CATT organization is initiated with as much experience behind it as possible. It is expected that completion of this task, from the identification of potential extension network members from known contacts and personnel lists to completion of the initial rounds of correspondence prior to the organizational meeting will take 3 months to complete.

Activity 2. Conduct the CATT Organizational Meeting.

After the CATT network membership has been identified and initial input has been received, the next task of this investigation is to hold an organizational meeting of the CATT. Member-selected representatives from each aquaculture region or state in Mexico will attend the organizational meeting along with representatives from US institutions. The primary purpose of the organizational meeting will be to establish the specific goals of the CATT program for aquaculture research, extension, and outreach, identify a CATT director, and establish an organizational communication structure. In addition, the role and structure of the 1st Annual Sustainable Aquaculture Technology Transfer Workshop will also be established at this time by CATT members and HC cooperators. The CATT Director will then assume the role as a HC Co-Principal Investigator.

Based upon results and feedback of the initial member correspondence, a meeting agenda will be established by the project team. Meeting preparations including workshop location, plan, membership, and schedule will be made to meeting representatives and the general CATT extension network. The meeting will be held in a central location, (e.g., Veracruz), in the summer of 2005 for approximately 2 days. The proposed project will cover travel and meeting organization costs for all attendees.

HC Co-Principal Investigators Cervantes Trujano and Perez-Sanchez will the play key roles in the preparation and holding of the CATT organizational meeting. US Co-Principal Investigators Jacob, Timmons, Baker, Rayburn, Schreibman, Fitzsimmons, Costa-Pierce, and Belcher will assist both in the preparation of and in the holding of the meeting. The CATT organization meeting will take place over 2 days in July 2005, however the preparation for the meeting and the compilation of information resulting from the meeting will take approximately 1.5 months.

Activity 3. Initiate CATT Program

The primary goal of the CATT is to provide increased flow of sustainable aquaculture production information to the existing or potential producer stakeholders. Details of how the CATT network is structured and functions will be developed by network members; however the general format of the program will be a virtual communication network between members that supports the request of information made either directly to the network or through a network member (extension agent). The CATT program will also work together as a single entity to identify research and extension goals within Mexico and depending upon additional government support, will administer research funding to support this research. The CATT will thus meet the two main activities of US SeaGrant (research and outreach).

The leading form of communication through which most information is transferred within New York's Cornell Cooperative Extension (CCE) program is its central website. The CCE website, parts of which are accessible to members only, is a clearinghouse of information for extension agents that are located throughout NY counties. Information available on this website includes contact information and specialty/role identification of all participating members, notification of active grant programs, extension success stories, copies of member research papers, employment enhancement information, a calendar of events, and relevant agriculture news updates. Similar websites that focus on information resources available according to subject area are operated by NY SeaGrant, Texas SeaGrant, and NY SeaGrant. A central CATT website and communication system similar to these websites will be designed to support the aquaculture extension network in Mexico.

Technology and production related information will be initially provided to production stakeholders via direct or member connection to the CATT extension network. Extension information provided through this project will mostly include electronic/website support and director office expense support (telephone, fax, and mail) for the distribution of oral or written materials and handbooks. As additional funds for this program are obtained, more proactive extension services such as in-field demonstrations and remote workshops will be provided by the CATT.

The director of the CATT will be identified in the organizational meeting approximately 3 months into the proposal. The HC PI will become the lead person coordinating the activities that support the center mission. Anticipated responsibilities of the CATT director include supervision of the website/information network, further identification of extension needs (information from research and government entities to producer stakeholders) and information dispersal. CATT activities are anticipated to be 30% of the director's time, which will be paid for (see Budget) through this project. US and existing host country co-principal investigators will provide assistance to the CATT director. Note that one of the HC Co-PI's may become the CATT Director.

Activity 4. Establish Ties with US Resources

One of the goals of the CATT will be to establish research and extension ties with US universities and institutions including the US SeaGrant System. The purpose is to strengthen the extension capability of the CATT program and to provide a basis for follow-on funding efforts. One activity of the CATT would be identification and English/Spanish translation of relevant sustainable aquaculture information. Potential US resource collaborators to complement their Mexican counterparts will be identified by the US Co-Principal Investigators based upon information determined by the CATT members. The initial relationship between US and Mexican counterparts will be one of research identification and information exchange. As funding is obtained, collaborative research and extension arising from these relationships is anticipated.

Project Schedule

Project Timetable	2005 Q2	2005 Q3	2005 Q4	2006 Q1	2006 Q2
Recruit key personnel as members of the CATT program,	X	X			
Determine status/needs of sustainable aquaculture extension					
Hold organization meeting of CATT		X			
Establish resource network objectives, structure, website	X	X	X	X	X
Establish ties with US Resources			X	X	X
Write Final Report (including Extension recommendations and CATT website)					X

Regional Integration

Improved aquaculture extension information exchange throughout Mexico is the focus of this project. In particular, this project seeks to unite and build upon aquaculture extension work that has been initiated recently (e.g., CRSP/USAID work with Pacific and Gulf of Mexico universities) and enhance these efforts under a singe extension theme of sustainable aquaculture development. In addition to disseminating technology to aquaculture development stakeholders from current CRSP/USAID projects, the CATT also intends to disseminate other Mexican and US based sustainable aquaculture research technology to stakeholders throughout Mexico. The host country co-principal investigators have established contacts with Mexican universities and government institutions on both coasts and have expressed a strong interest in pursuing extension related activity.

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Diversifying and Strengthening Aquaculture Extension Capacity to Develop a Regional Extension Service Model

Applied Technologies and Extension Methodologies 6 (11.5ATE6)/Activity/Mexico

Investigators

Maria Haws	US Principal Investigator	University of Hawaii, Hilo
John Supan	US Principal Investigator	Louisiana State University
Eladio Gaxiola	HC Principal Investigator	Universidad Autonoma de Sinaloa, Mexico
Emilio Ochoa	HC Principal Investigator	Ecocostas, Ecuador
Pamela Rubinoff	Collaborator	University of Rhode Island

Objectives

The overall objective is to enhance the effectiveness of extension efforts and build specific technical skills to improve outcomes related to health and aquaculture in Western Mexico with LAC-wide applicability. This activity will be a shared activity with resources and personnel partially sponsored by the David and Lucille Packard Foundation project, "Best Management Practices for Shrimp Culture in Sinaloa". This project aims to build capacity to mitigate potential social and environmental impacts of shrimp culture, improve efficiency of shrimp production particularly for cooperative producers and diversify aquaculture to offer alternatives to shrimp culture for coastal residents, including women and cooperatives.

The capacity building efforts will continue the work begun in Tenth Work Plan when an international exchange for extension training was held in Mazatlan, Mexico. This event provided training to 75 professionals and community leaders from Pacific Mexico and several CRSP countries (UJAT/Mexico, Ecuador, Nicaragua, and Honduras) in extension methods and tools. The need for a multi-institutional extension delivery system to support aquaculture development and to address specific impediments has been recognized by the major stakeholder groups involved in aquaculture in Pacific Mexico. The purpose of the first extension training event was to build capacity among professionals in Mexico and work towards developing an extension model similar to the US Land Grant and Sea Grant programs. The training included topics in extension theory, concepts and tools to a wide range of professionals and community leaders that were involved with aquaculture development in a wide variety of ways. It also provided an opportunity for extension and community development professionals from other sectors such as agriculture and public health to share lessons learned and methodology with aquaculture professionals, who in turn were able to raise awareness of the benefits that aquaculture offers to rural communities among the other professionals.

The skills developed as a result of this work have been very helpful in improving the ability of aquaculture professionals to work in coastal communities and learn from other extension professionals. It has also helped connect leading scientists, extension agents and community-members so that on-going research results are more rapidly disseminated and applied in the field. Furthermore, it has strengthened the integrated approach favored by the researchers and extension agents involved in aquaculture and community development and has begun laying the foundation for a multi-institutional extension effort. This has benefited not only the aquaculture development efforts undertaken by members of this group, but also their conservation, community development and gender equity initiatives. The professionals and institutions involved in this effort (UAS, CI, CESASIN, CREDES, ISA) are engaged in a wide range of resource management activities.

The training proposed for the Eleventh Work Plan will advance these efforts by providing additional training and reinforcement to the same group of professionals to allow them to continue improving their extension skills. This training will targeted towards transfer of specific extension tools and approaches. These will include topics such as planning extension campaigns and strategies, adult learning methods, use of media, action-research and demonstration methods, production of extension materials (brochures, posters, booklets), science for management and gender issues. In addition, intensive technical training will be provided to address priority issues identified in Tenth Work Plan as necessary to improve the health related benefits of aquaculture and reduce impacts such as farm sanitation, food sanitation, handling, processing, water quality and aquaculture and small-scale marketing. This training will be conducted in a Training-of-Trainers mode.

For the second training event, professionals trained in the Training-of-Trainers course will replicate the training to other professionals, producers and community members as a means of replicating the benefits of this capacity building. This second group will also be closely involved in the site visits and routine extension work conducted by UAS. This will also serve to reinforce the skills learned in extension and community outreach acquired by the core group of trainers, extension agents and researchers involved in this work. Additionally, these individuals will be involved in planning and working sessions related to Investigations 2 and 3 as an additional means of expanding their awareness of the issues and increasing their skills for these topics.

Specifically, this initiative will:

1) Provide training and practical experience in extension methods, tools and models during two events:
2) A Training-of-Trainers event including advance extension methods and technical skills related to health aspects of aquaculture; and
3) A producer/stakeholder training event to disseminate information and methods to producers and allow Trainers to begin replicating the first training event as their own skills are reinforced.
    - The technical areas identified as critical in Tenth Work Plan which will be focused on at the training events are: advanced extension tools and methods; bivalve sanitation; water quality monitoring; aquatic product handling and sanitation, farm-level sanitation practices; methods for working with marginalized groups including the physically-disabled, marketing, value-added strategies and transportation.
    - Training will be provided in marketing and value-added strategies that increase revenues and their interaction with sanitation issues by Quentin Fong, Fisheries Industry Technology Center, University of Alaska Fairbanks-Kodiak who has volunteered his time for this activity.
4) Provide extended opportunities for mentoring from Sea Grant and Land Grant specialists.
5) Work with a range of local partners towards developing multi-institutional partnerships and arrangements which together make up a regional extension program for Pacific Mexico (Naryarit, Sinaloa and Sonora).
6) Impart specific technical knowledge and skills in topics related to human and environmental health
7) Production of training modules in Spanish and English for extension methods and the technical topics to be widely distributed among workers in these fields. Materials will be produced in printed form, on CD's and posted to the websites of the participating institutions.
8) Four Host Country students will be involved in this work-Roberto Quintana (Mexico, LSU), Abelardo Rojas (Nicaragua, UHH) and two students to be identified from UAS in Mexico.

Significance

Previous work in Pacific Mexico has demonstrated that a major obstacle to further aquaculture development, particularly for species other than shrimp, is the weakness or lack of extension capacity in rural areas, particularly in the more inaccessible coastal areas. Pacific Mexico has extensive areas of wetland and bays which host small communities which receive few social services, yet have great need for economic alternatives to fishing other than participation in the drug smuggling activities which operate fairly freely along the coast. There is also a critical need for improved institutional cooperation in order to bring together the strong research capacity of the Mexican scientific community with extension agents and community development workers. Mexico is also the site of numerous international development and conservation efforts and a general deficit of communication and cooperation between the various efforts which also plays a role in hindering efforts to improve the benefits derived from aquaculture, particularly in the areas of health, sanitation and socio-economic aspects.

Advances towards building capacity and bringing together the various sectors involved in community development were made during the Tenth Work Plan efforts which included the first Training-of-Training Extension workshop. This was the formal first extension training that many of the scientists and extension agents had received. Additionally, because extension professionals from non-aquaculture sectors such as public health, agriculture and community-development were included, the event provided an opportunity for the participants to learn different methods from each other as well as for health and technical specialists to plan and share expertise for the series of three case studies on various aspects of human and environmental health aspects of aquaculture. The work proposed for Eleventh Work Plan will both enable professionals supporting aquaculture to continue improving their skills and provide a venue to link with community members and other non-technical stakeholders for joint planning to support health and aquaculture efforts.

Quantifiable Anticipated Benefits

Target Groups: Aquaculture extension workers and researchers in Pacific Mexico; key private sector representatives; oyster growers in Nayarit, Sinaloa and Sonora; Women's oyster culture cooperatives of Nayarit; Women's oyster culture cooperative of Puerto Peñasco; Conservation International; Universidad Autonoma de Sinaloa (UAS, Culiacan and Mazatlan Campuses); Sinaloa Institute for Aquaculture (ISA); Ecocostas, an NGO dedicated to conservation and sustainable development for Latin America; State Committee for Aquatic Sanitation (CESASIN); and the Federation of Shrimp Cooperatives. Linkages will also be made to the NOAA International Sea Grant efforts through participation of Maria Haws, John Supan and Quentin Fong, Sea Grant personnel/associated faculty from Hawaii, Louisiana and Alaska. Four Host Country students will be involved in this work and will benefit from the hands-on learning experience.

Quantifiable Benefits: Demonstrated increases in aquaculture extension skills; increased knowledge of extension and outreach practices used in other fields; increased effectiveness in transferring practices that increase benefits from aquaculture that enhance human health; increased skill levels for improving areas of health and sanitation related to aquaculture; improved knowledge of the linkages between the environment and health; and identification and development of multi-institutional strategies and agreements that can be implemented in the future to increase human health benefits.

Activity Plan

Two training events will be held in Mazatlan, Mexico. The first event will be held as a Training-of-Trainers course and will be similar to the first International Extension Exchange and Training event held in June 2004. Professionals already working to support aquaculture development and associates from other fields working in the same communities will continue learning extension tools and methods as well as specific technical fields related to health and aquaculture. Mexican and international specialists will jointly design the curriculum and provide training. The international specialists to participate are Emilio Ochoa, Extension specialist; John Supan, Bivalve culture and sanitation specialist; and Maria Haws, tropical mariculture specialist. Quentin Fong will provide training in the aspects of aquaculture development related to marketing, value-added strategies and the relationship with sanitation. This contribution is viewed as important since the Tenth Work Plan work highlighted issues associated with sanitation and quality as well as generally poor marketing ability as being major impediments to increasing sales and revenues. Fong will also serve as the liaison with the team of food product development and sanitation specialists at the University of Alaska Fairbanks-Kodiak's Fisheries Industry Technology Center. It is hoped this tie will help establish future exchanges and learning opportunities between the Mexican and the Alaskan partners. Fong will serve in this capacity pro bono and has also offered to pay his own travel expenses. Four graduate students will also be involved in this effort: three Mexican nationals (2 at UAS and one at LSU) and one Nicaraguan national (UHH). The latter individual is also involved in joint efforts of URI and UHH to develop bivalve sanitation plans in Nicaragua and Ecuador under separate funding from USAID and participation in this work will help build capacity in these countries as well.

It should be noted that aquaculture specialists from the UJAT CRSP project will be invited to attend the training although funding is not requested in this proposal for their expenses. Mr. Ulises Hernandez of

UJAT participated in the first Training-of-Trainers event in June 2004 as a co-Trainer. His contribution to the training was outstanding and continuation of ties between the two Mexico projects is viewed as very productive.

A second training event will be held with a target audience of producers and community leaders. The trainers trained in the Tenth Work Plan and the first training event of the Eleventh Work Plan work will design and conduct the training. The international specialists will not directly participate in this, but will provide support to the design of the training materials and by helping with linkages to the extension work to be conducted in the communities. International specialists will also assist with reviewing and editing the training materials for their final publication.

During the visits of the international specialists for the first Training-of-Trainer event, they will also travel to the communities that were the focus of study during the Tenth Work Plan work to provide short training sessions and technical assistance to producers, processors or vendors of tilapia, oysters and shrimp. This will also serve to familiarize the international specialists who have not traveled to this region previously with local aquaculture operations so that they may be better able to assist the Mexican experts in preparing the producer/community training events and extension activities.

Regional and Global Integration

This work is a continuation of the Tenth Work Plan work to build extension capacity and is linked to conservation and sustainable development initiatives undertaken by URI, UHH, UAS, Conservation International and CESASIN in the Pacific states of Sonora, Sinaloa and Nayarit. This includes the Santa Maria Bay (BSM) management initiative, management of the Marismas Nacionales (wetland systems in Nayarit), development of best management practices for shrimp culture (David and Lucille Packard Foundation) and aquaculture development efforts by the above mentioned institutions. Additionally, this work will contribute to a series of international extension training efforts to be undertaken for a five year period by the USAID/EGAT-sponsored SUCCESS project (Sustainable Coastal Communities and Ecosystems) in other CRSP regions such as Nicaragua, Ecuador, and East Africa.

Schedule

Starting Date: 1 June 2005.
End Date: 30 June 2006.

Task	Month												
	J	J	A	S	O	N	D	J	F	M	A	M	J
Design training curriculum for TOT	X												
Logistical preparations for TOT	X												
TOT course held in Mazatlan		X											
Design of community-based curriculum		X											
Logistical preparations for community course		X											
Community training course held in Mazatlan			X										
Publication of final training modules					X								

Site Visits and Information Exchange on Cichlid Culture and the Adoption of Aquaculture CRSP Technologies in Aquaculture CRSP Host Countries

Applied Technology and Extension Methodologies 7 (11.5ATER7)/Activity/Mexico, Honduras, Philippines, Thailand, Kenya

Investigators

Amrit Bart	HC Principal Investigator	Asian Institute of Technology, Thailand
Remedios Bolivar	HC Principal Investigator	Central Luzon State University, Philippines
Wilfrido Contreras	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Nancy Gitonga	HC Principal Investigator	Kenya Fisheries Department
Daniel Meyer	HC Principal Investigator	Pan American School of Agriculture, Honduras

Objectives

1) Strengthen the human resources at host country institutions of the Aquaculture Collaborative Research Support Program (Aquaculture CRSP) through direct exposure to research and extension activities at other Aquaculture CRSP sites.
2) Strengthen the networking capabilities among the Aquaculture CRSP host country institutions working with cichlid culture.
3) Organize and implement short courses, seminars and classroom presentations that include information on some aspects of cichlid culture that have been derived from Aquaculture CRSP supported efforts around the globe.
4) Develop concepts for joint future research and extension proposals between and/or among the Aquaculture CRSP host country institutions working on cichlid culture.

Significance

The training of extension agents, fish farmers and students is considered a fundamental component of Aquaculture CRSP activities throughout the world. The need to deliver timely information and appropriate technological packages to end-users is critical for the development of sustainable aquaculture activities.

Aquaculture CRSP sponsored training workshops are a proven and effective means to achieve these goals. Through workshops, researchers obtain feedback from participants useful for identifying and prioritizing future research efforts. According to the Aquaculture CRSP Training Plan: Perspective, Experience and Directions, the training of technicians, farmers and extension agents can be the most effective way to disseminate research results and good management practices in aquaculture (Bolivar et al., 2002).

Unfortunately, much of the knowledge, technologies and experiences generated by the Aquaculture CRSP host country institutions have remained largely within the borders of each country and little effective information exchange has occurred among the several countries. Most information exchange takes place at the Aquaculture CRSP annual meetings and the adoption of new techniques and experiences has remained at the regional scale.

To overcome the inherent problems related to global distances and difficulties with communications, this proposal intends to train trainers *in situ*, facilitating travel of host country PIs, collaborators and students. This strategy will reduce the time needed to produce training manuals, proposals or publish research results and allow for a better understanding of the techniques being developed for cichlid culture at each Aquaculture CRSP host country institution.

Strengths of Participating Institutions:

Panamerican Agricultural School, Honduras: The Panamerican Agriculture School, also known as Zamorano, is a private, non-profit, US corporation operating in Honduras. The Zamorano student body of

850 is drawn from 18 countries from throughout Latin America. Zamorano has had an active outreach program for more than 20 years and the institution has outstanding facilities and human resources for applied research and organizing and providing training to students, farmers, extension agents and other interested individuals or groups.

The Zamorano Aquaculture Station, founded in 1976, has had an important role in both formal and informal training of individuals interested in fish and crustacean farming in Latin America. Zamorano's research efforts in aquaculture have focused on studying and developing efficient techniques to manage production systems, reduce feed inputs and the environmental impacts derived from aquaculture operations, and evaluating genetic stocks for use in the region. We have developed a series of manuals and a web site in Spanish to facilitate information exchange and promote the culture of tilapia and other species regionally. Our station has excellent infrastructure for training and research activities in aquaculture with ponds and tanks (total > 100 units), laboratories for water and microbiological analysis of samples, modern classrooms and office space. We have been actively involved in fish culture extension in Central America for more than 15 years. We have received support for our outreach activities from many donors, including the GTZ and DSE of Germany, Public Welfare Foundation and USAID, NOAA-Sea Grant, and others. In collaboration with other agencies and interested groups we routinely organize field-days, short courses and seminars on multiple topics related to successful fish and shrimp culture.

Central Luzon State University (CLSU), The Philippines: The CLSU houses the Freshwater Aquaculture Center (FAC) which is a multidisciplinary research unit of the university. FAC is responsible for aquaculture and fisheries research and development through close collaboration with the College of Fisheries, with which FAC shares a core research staff and physical facilities. Current research topics at FAC include tilapia genetics, water quality management, fish health management, fish nutrition, fisheries economics, aquaculture systems and aquatic ecology. The Aquaculture CRSP funded research at FAC focuses on evaluating tilapia feeding strategies in semi-intensive culture systems and surveying management practices on private tilapia farms.

The establishment of the Tilapia Science Center (TSC) at CLSU is in recognition of the outstanding achievements and significant contributions made by the institutional partners to promote the growth and development of the tilapia industry. The TSC is a unique partnership of agencies and institutions representing academe (CLSU), the public sector (Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center), a non-profit NGO (GIFT Foundation, International, Inc.) and a private company (FISHGEN, Inc.). This strategic alliance combines innovative education with research, extension and entrepreneurship to effectively improve the quality of life of tilapia farmers in particular, and of other people in general. These institutions are located in the vicinity of the FAC.

Facilities of the FAC include research laboratories for water quality, aquatic biology, fish pathology and fish nutrition, experimental ponds, tanks, hapas and aquaria. The FAC has a living museum intended to showcase a living collection of indigenous freshwater fishes from the Philippines.

Universidad Juárez Autónoma de Tabasco (UJAT), Mexico: The Laboratory of Aquaculture (LA) at UJAT has been addressing the problems that small farmers face in the region by: 1) developing masculinizing systems for tilapia and native fish which are safe for fish farmers and the environment, 2) producing a new line of tilapia for brood-stock replacement, 3) diversifying aquaculture practices using native species, 4) implementing polyculture alternatives for shrimp farmers, and 5) conducting regional workshops on safe handling of steroids and sex inversion techniques.

An important aspect of all projects at UJAT has been the incorporation of an extension program. The primary results that can benefit other institutions are: a. development of a filtration system that eliminates MT from intensive masculinzation ponds; b. develop-ment of a methodology to sex inverse larvae of carnivorous species of fish using *Artemia nauplii* as vehicle; c. effective masculinization of two native cichlids and feminization of the tropical gar; d. selection of a tilapia line that performs better than the brood-stock traditionally used in Tabasco. The LA has produced videos, pamphlets and written materials that may be helpful to other countries. UJAT will benefit from other institutions that are currently working on reproduction and grow-out systems, genetic line selection and extension methods.

Asian Institute of Technology (AIT), Thailand: The AIT hosts the Aquaculture and Aquatic Resource Management (AARM) Program, an academic and research program focused on improving regional institutional capacity through innovative approaches that integrate education, research and outreach. AIT is a research hub for the region.

Three cross-cutting research themes are currently emphasized in aquaculture within AARM: 1) small-scale aquaculture, 2) seed production and genetics, and 3) fish nutrition and feed management. AIT has extensive pond facilities, water chemistry, nutrition and genetics laboratories, a hatcheries and a team of 30 professionals.

Some ongoing CRSP supported research activities at AIT include: innovations in *Macrobrachium* culture in recirculating systems; fertilization regimes for ponds with Nile tilapia receiving supplemental feeds; use of rice-straw as a resource for freshwater pond culture; polyculture of lotus and snakehead for recycling wastewater from intensively fed ponds; reproductive performance and growth of improved tilapia (*Oreochromis niloticus*); use of ultra-sound in immersion protocols for efficient sex-reversal of Nile tilapia; reproduction of *Spinibarbus denticulatus*; and impact of tilapia introductions on native indigenous species. AIT provides training, study tours and has an extensive network of farmers, entrepreneurs and academic/research institutions to partner with for the dissemination of research results and other technical information.

Fisheries Department (FD), Kenya: The Sagana Fish Culture Farm has served as the Kenya FD principal warm-water research, educational and seed supply center since 1948. Currently there are 113 ponds of different sizes that are used for research and fish production. Part of the site has been integrated with ongoing dairy, agro-forestry and poultry programs. Aquaculture CRSP activities, such as in-service training in aquaculture for the Fisheries Extension staff, have involved collaboration between the FD, Moi University and Oregon State University.

Research topics at Sagana Fish Culture Farm and related Aquaculture CRSP activities include:

1) Determination of the performance of three strains of tilapia (*Oreochromis niloticus*);
2) Determination of the most cost-effective fish feed combination for tilapia culture;
3) Cage culture of tilapia using low-input feeding regimen in static water ponds;
4) Training of Fisheries Extension Staff in construction and management of fish ponds, and enterprise budgets; and
5) On-farm trials of selected pond management techniques.

The Sagana Fish Farm is involved in the recruitment of an indigenous species (*Labeo* sp.) as a potential candidate for farming. Documentation on our experiences in semi-intensive, low-input systems of tilapia culture is available. A manual for aquaculture trainers is being drafted, arising from training of fisheries front-line staff between 1999 and 2003. Additionally we are organizing a Documentation Center to facilitate dissemination of information to interested parties.

Quantified Anticipated Benefits

Direct target groups of this activity will be:

- Aquaculture CRSP HC PIs will gain valuable first-hand knowledge of the physical installations, research and extension activities and functioning of other Aquaculture CRSP HC institutions working to promote cichlid culture in other regions of the world.
- Staff and students at each hosting institution will benefit from interactions with the visiting PIs and should gain new knowledge and insight into cichlid culture in other parts of the world.
- The visits will provide opportunities for strengthening existing linkages as well as for making new professional contacts, leading potentially to a greater degree of networking among researchers in Aquaculture CRSP host-country institutions around the globe and the possible development of future collaborative efforts.

Indirect target groups of this activity will be:

- Fish farmers from the host-countries will be better served thru extension and research provided by institutions with support from the Aquaculture CRSP
- Students at host-country institutions will benefit from their professors enhanced knowledge and understanding of the techniques and physical installations used for culture of cichlid in other parts of the world

Activity Plan

Host country personnel will travel to other Aquaculture CRSP sites within the cichlid group. PIs hosting each site visit will organize a workshop to be held for the benefit of the visiting PIs. The main purpose of the workshop is for the host PI and his/her colleagues to present cichlid culture work going on in the host country, focusing particularly on Aquaculture CRSP efforts and Aquaculture CRSP technologies that have been successfully implemented, so that comparisons of these can eventually be made across all sites. This will complement the results of the survey to be conducted under Investigation 2 of this proposal. However, each visiting HC PI will, as a minimum, do the following:

- Participate in the workshop to be organized by the host PI, giving a short presentation describing work on cichlid culture and the results of recent research and extension efforts being conducted in their own home country and highlighting any Aquaculture CRSP technologies that have been implemented.
- Participate in field visits to in-country research stations, university laboratories and local fish farms; and
- Meet with the local host country institution officials to discuss Aquaculture CRSP related activities and possible areas for future interactions.

Upon returning to his/her home country, each Aquaculture CRSP researcher will:

- Give a seminar/presentation to coworkers in his/her home institution to share the findings of the visits to the other sites.
- If possible, give additional seminars to share the findings of the site visits with colleagues in collaborating institutions either in the home country or in the region
- Incorporate information, technologies and experiences from the countries visited into training materials, publications and future research protocols.
- Place any written or electronic materials obtained in the site visits in appropriate libraries or other types of collections available to students and the general public at their home institution.

Deliverables:

- A compendium of all presentations, handouts, or other written information provided to the visiting PIs and other participants in the workshops to be conducted under Investigation 1;
- A report on Aquaculture CRSP "best practices," based on the results of the survey (Investigation 2) and the workshops and site visits (Investigation 1), prepared with the assistance of the PMO; and
- One "Echo-Seminar" at each site, to be presented by the HC PI to his/her home institution colleagues upon completion of the visits to the other four sites.

Schedule

- The proposed visits of HC-PIs to other Aquaculture CRSP sites will take place following the completion of the survey described as Investigation 2 of this proposal, most likely between June 2005 and February 2006. A tentative schedule for the site visits is shown in Table 1. The plan is for each HC PI to visit every site, with all PIs present at each site at the same time. To keep travel costs as low as possible, travel to the Philippines and Thailand will be planned as a single trip, as will travel to Mexico and Honduras. Changes to this schedule may be made if necessary.
- The workshop compendiums will be compiled by the hosting PIs immediately following each workshop/site visit.
- Work on the "Aquaculture CRSP Best Practices" report will begin following the first site visit and be completed within three months of the final site visit, or by 30 April 2006, whichever comes first.

- "Echo-Seminars" will be presented at all home institutions within three months of the final site visit (tentatively scheduled for January 2006).
- The incorporation of any written and/or other types of materials on cichlid culture acquired on the visits into public collections and used in teaching at the different institutions will be done following each site visit.

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Comparison of the Implementation of CRSP Technologies in Five Aquaculture CRSP Host Countries

Applied Technology and Extension Methodologies 8 (11.5ATE8)/Study/Mexico, Honduras, Thailand, Philippines, Kenya

Investigators

Amrit Bart	HC Principal Investigator	Asian Institute of Technology, Thailand
Remedios Bolivar	HC Principal Investigator	Central Luzon State University, Philippines
Wilfrido Contreras	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Nancy Gitonga	HC Principal Investigator	Kenya Fisheries Department
Daniel Meyer	HC Principal Investigator	Pan American School of Agriculture, Honduras

Objective

1) Compare the adoption of Aquaculture CRSP technologies by cichlid producers in the five participating Aquaculture CRSP host countries.

Significance

The present group of Aquaculture CRSP researchers is in a unique position to compare and evaluate the culture of tilapia and other cichlid species, and particularly the implementation of Aquaculture CRSP technologies, in several important areas of the world. Regional differences in the species being cultured, variety and costs of local inputs, and management protocols vary among the several regions where the Aquaculture CRSP has supported aquaculture development activities.

Quantified Anticipated Benefits

Direct target groups of this study will be:

1) The five Aquaculture CRSP host-country researchers will acquire a better understanding of the cichlid species being cultured, management protocols, and inputs used in other parts of the world.
2) Students in the host-country institutions will benefit from the enhanced knowledge and understanding that local host-country researchers will attain from their direct participation in this study.
3) A final report comparing the techniques utilized on small-farms for cichlid culture in each of the five participating host countries will be prepared based on the questionnaire developed by the HC researchers.

Indirect target groups of this study will be:

1) Fish farmers in each of the Aquaculture CRSP host countries will benefit by having available more knowledgeable professionals to provide advice and direct effective research at local universities and NGOs.

Activity Plan

The Aquaculture CRSP host country researchers will develop a questionnaire to assist in obtaining and organizing technical information to describe and compare the implementation of Aquaculture CRSP technologies in each host country. The questionnaire will be filled out by the PIs themselves, prior to visiting each others' sites, and the results will provide a foundation for discussions and information exchange that will take place during the subsequent site visits. A draft of the questionnaire being developed for this activity is attached to this proposal.

Wherever possible, PIs will provide photographs illustrating the implementation of CRSP technologies on private farms or at government or research sites in their respective countries.

One HC researcher will be designated to compile and analyze the information from the questionnaires from all countries as part of the final report of this activity. The results of this study will be summarized in a Aquaculture CRSP final report and disseminated via newspaper articles, presentations at scientific/technical meetings and university-level classroom instruction, as appropriate.

Deliverable: The main deliverable from this activity will be a report summarizing the results of the survey.

Schedule

- The text of the survey questionnaire will be finalized by 1 April 2005.
- The HC PIs will complete the questionnaire and submit their responses by 15 April 2005.
- The survey responses will be analyzed and summarized, and a draft report completed by 13 May 2005.

Special Sessions, Travel, and Poster Awards at 2005 and 2006 World Aquaculture Conferences and Aquaculture America–2006

Applied Technology and Extension Methodologies 9 (11.5ATE9)/Activity/Philippines

Investigators

Kevin Fitzsimmons	US Principal Investigator	University of Arizona
Remedios Bolivar	HC Principal Investigator	Central Luzon State University

Objectives

1) Organize special sessions at the 2005 (Bali) and 2006 (Florence) World Aquaculture Society Meetings. These sessions would focus on Sustainable Aquaculture Research conducted in Aquaculture CRSP host country institutions.
2) Provide travel support for three or more international contributors from Aquaculture CRSP country institutions to attend the 2005 and 2006 World Aquaculture Conferences.
3) Provide three student travel awards to present Aquaculture CRSP results and three poster awards to recognize research that addresses goals of sustainable aquaculture in developing countries at 2005 WAS-Bali and 2006 WAS-Florence meetings and the 2006 Aquaculture America meeting in Las Vegas, NV.

Significance

The Aquaculture CRSP will sponsor three special sessions focused on research conducted by Aquaculture CRSP scientists. The conference in Bali, Indonesia, scheduled for 9–13 May 2005, will focus on aquaculture in the developing countries of South and Southeast Asia. Many of the Aquaculture CRSP host country institutions will be heavily represented at the meeting. Findings from this research will benefit fish producers in developing and developed countries. The focus of the session will be the use of tilapia in developing countries for sustainable production systems.

The Aquaculture America 2006, 13–16 February, is the primary aquaculture conference in the US each year. We will organize a special session that will focus on results of Aquaculture CRSP research and their application to tilapia aquaculture in the US.

The conference in Florence, Italy, 9–13 May 2006 will be heavily attended by European donor agencies and scientists working with these agencies in developing country aquaculture. The specific session in Florence has been titled "Sustainable Aquaculture-Linking Tradition with Technology – Aquaculture CRSP 2006".

Travel support is critical to the ability of scientists from developing countries to present their findings in an international forum. Host country CRSP scientists benefit from the opportunity to discuss their work amongst themselves, with their US colleagues as well as the rest of the international community. Students often need travel support to present their research. Students should also be recognized for the tremendous work they put into poster presentations at professional meetings. These are often the first opportunity students have to meet with the wider scientific community and have a chance to discuss their findings with many of the experts.

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

Quantified Anticipated Benefits

Target Groups:

1) Host country scientists.
2) Our target group is the international community devoted to development of sustainable aquaculture technologies in developing countries.

Quantifiable Direct Benefits from the Conference:

1) Have a special session at the World Aquaculture Meetings that presents a comprehensive overview of the research contributions of the Aquaculture CRSP.
2) Ensure a strong participation of Aquaculture CRSP host country PI's in the World Aquaculture meetings.
3) Present and publish a large body of information generated by Aquaculture CRSP researchers.
4) Provide opportunities for students to present their work during oral or poster sessions.

Quantifiable Indirect Benefits from the Conference:
1) Establish relationships between CRSP research community and international aquaculture scientists, producers, and development community.
2) Have a strong program with informative presentations and good networking.
3) Assist development of professional careers of students.

Research Design or Activity Plan
Location of Work:
Aquaculture America 2006 – Arizona and Las Vegas, NV.
WAS 2005 – CLSU, Arizona and Bali, Indonesia.
WAS 2006 - CLSU, Arizona and Florence, Italy.

Methods: Fitzsimmons has already been asked by the program committee planning the Bali conference to organize the tilapia session. The Bali session has been approved and is listed in the initial program and call for papers. Fitzsimmons serves on the program committee for the Florence meeting and accepted a request from the other committee members to develop a session recognizing the Aquaculture CRSP as the group most closely matched to the conference overall theme of linking tradition with technology.

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

The committee will also evaluate student abstracts to determine student travel awards based on the quality of the work and its applicability to the Aquaculture CRSP goals. At the conferences, committee members will also evaluate student posters and provide award accordingly.

Proposed titles of special Aquaculture CRSP conference sessions include:
Special Session: "Tilapia and Contributions to Sustainable Aquaculture – Aquaculture CRSP 2005"
Special Session: "Aquaculture CRSP and Tilapia Aquaculture Contributions – Aquaculture America 2006:
Special Session: "Sustainable Aquaculture-Linking Tradition with Technology – Aquaculture CRSP 2006"

Regional and Global Integration
1) Local participation on organising committee.
2) Regional participation on organising committee.
3) International participation on organising committee.
4) Local, regional and international co-sponsors and vendors for trade show.
5) Strong international representation in papers presented.

Schedule
Year 1:
Jan. 2005 – Call for Bali abstracts, Announce student poster awards for Bali conference.
Mar. 2005 – Invite Aquaculture CRSP host PIs to apply for travel support.
Mar. 2005 – Organise selection committee.
Mar. 2005 – Receive and edit papers.

Mar. 2005 –Select and invite scientists who will receive travel support.
Mar. 2005 – Determine travel plans for those receiving travel support, submit visa requests.
Apr. 2005 – Provide travel stipend for airfare, registration, and other travel costs.
9–13 May 2005 – WAS Annual Conference – Bali, Indonesia.
June 2005 –Prepare yearly report

Year 2:
Sept. 2005 – Call for abstracts.
Nov. 2005 – Invite Aquaculture CRSP host PIs to apply for travel support.
Dec. 2005 – Re-organise selection committee (if needed).
Jan. 2006 – Receive and edit papers.
Feb. 2006 – Judge student posters at meeting in Las Vegas, present awards at conference.
13–16 February 2006 – Aquaculture America Conference – Las Vegas, Nevada.
Feb. 2006 –Select and invite scientists who will receive travel support
Mar. 2006 – Determine travel plans for those receiving travel support, submit visa requests
Apr. 2006 – Provide travel stipend for airfare, registration, and other travel costs
9–13 May 2006 – WAS Annual Conference – Florence, Italy.
June 2006 –Prepare project final report.

Literature Cited

http://www.was.org - Program and call for papers website

Aquaculture CRSP Sponsorship of the Seventh International Symposium on Tilapia in Aquaculture

Applied Technology and Extension Methodologies 10 (11.5ATE10)/Activity/Mexico

Investigators

Kevin Fitzsimmons	US Principal Investigator	University of Arizona
Wilfrido Contreras Sanchez	HC Principal Investigator	Universidad Juarez Autonoma Tabasco

Objectives

1) Provide support for Wilfrido Contreras and three international contributors from other Aquaculture CRSP countries to prepare papers that will be reported and published at ISTA 7.
2) Employee a UJAT student to assist with the compilation of papers submitted to the ISTA 7 conference proceedings.
3) Publish and print the Proceedings of the 7th ISTA for distribution at the actual conference.

Significance

The ISTA meetings traditionally were held every 4 years and are the premier international meeting focused directly on tilapia aquaculture. However, after the Manila meetings the demand for more information and number of other locations vying to host additional conferences led us to decide to hold the next ISTA within two years. In the past the ISTA's have provided one of the most important outlets for publication and discussion of the findings of Aquaculture CRSP supported research. Aquaculture CRSP has been a co-sponsor of the last three ISTA's (Fitzsimmons, 1997; Fitzsimmons and Carvalho, 2000; Bolivar, Mair, and Fitzsimmons, 2004). Aquaculture CRSP institutions in Mexico will be hosting ISTA 7 and we will be especially interested to ensure the success of the conference.

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

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

Quantified Anticipated Benefits

Target Groups:

1) Host country scientists.
2) The international community devoted to tilapia aquaculture.
3) Partial financial support for a graduate student to assist with conference planning.

Quantifiable Direct Benefits from the Conference:

1) Organize and host the Seventh International Symposium on Tilapia in Aquaculture.
2) Ensure a strong international participation in ISTA 7.
3) Present and publish a large body of information generated by Aquaculture CRSP researchers.

Quantifiable Indirect Benefits from the Conference:

1) Establish relationships between tilapia research community and Mexican farmers.
2) Have a strong program with informative sessions and good networking.

Activity Plan

Location of Work: ISTA 7 - UJAT and Guadalajara Conference Center.

Methods: Fitzsimmons and Contreras are on the organizing committee planning the ISTA 7 symposium. A sub-committee will be formed to set the selection criteria and then determine which applicant scientists will be awarded the preparation support. The selection criteria will be based on contributions of papers to the conference, past participation in Aquaculture CRSP projects and other available support. If partial support can be generated from other sources, the funds may be split to support additional participation.

A graduate student from UAT will receive assistance to assist with the conference organization. Specifically, the student will work with the publication committee working on the proceedings.

Regional and Global Integration

1) Local participation on organizing committees.
2) Regional participation on organizing committees.
3) International participation on organizing committees.
4) Local, regional and international co-sponsors and vendors for trade show.
5) Strong international representation in papers presented.

Schedule

June 2005 - Local organizing committee meets, First call for abstracts.
July 2005 - Select printer.
October 2005 – Begin to organize sessions.
May 2006 – Begin to receive and edit papers.
May 2006 –Select and invite scientists who will receive grant support to prepare their papers.
June 2006 – Determine travel plans for those receiving preparation support.
June 2006 – Provide stipend for preparation, and registration costs.
June 2006 – Submit final report.
September 2006 – ISTA 7.

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Evaluation and Improvement of Tilapia Fingerling Production and Availability in Honduras

Seed Stock Development and Availability 1 (11.5SDA1)/Study/Honduras and Dominican Republic

Investigators

Joseph J. Molnar	US Lead Principal Investigator	Auburn University
Daniel E. Meyer	HC Principal Investigator	Panamerican Agriculture School, Zamorano

Objectives

1) Evaluate the actual production costs and returns for producing tilapia fingerlings in Honduras.
2) Train NGO extension agents and fish farmers from several Latin American countries on the techniques of tilapia reproduction, sex-reversal and distribution of fingerlings.
3) Produce training materials in Spanish on the topics of tilapia reproduction and fry production and distribution, for dissemination to local farmers, NGO extension agents and other persons interested in tilapia culture.

Significance

The availability of good quality seed is often a factor limiting aquaculture development (Aquaculture CRSP, 2002, Egna and Boyd, 1997). The lack of adequate supplies of all-male tilapia fingerlings has been identified as a principal limiting factor to small-scale fish culture development in Honduras (Meyer, 1988; Triminio, 2001). Procuring reliable supplies of high quality seed for stocking local and remote sites is critical to continued development of the industry. A better understanding of the factors that can contribute to stable seed stock quality and quantity for aquaculture enterprises is essential. The purpose of the proposed activities is to understand the production costs and returns for producing and distributing tilapia fingerlings in Honduras, develop training materials in Spanish on tilapia reproduction and fingerling production that will be used in providing technical training to extension agents and fish farmers from several regional countries.

The production of cultured tilapia in Honduras will surpass 16,000 metric tons in 2004. Most of this production will be from two large commercial farms that supply fresh fillets to North American markets. The large commercial farms are self-sufficient in tilapia fingerling production. They occasionally sell excess fingerlings to small-scale producers.

Cultured tilapia for distribution in domestic markets is estimated at approximately 2,000 metric tons for 2004. Much of this production comes from small and medium-scale producers located throughout Honduras. The popularity of tilapia is increasing throughout Central America and several Honduran farmers are exporting fish to Guatemala and El Salvador.

Small and medium-scale Central American fish farmers often have difficulties in obtaining tilapia fingerlings for stocking their ponds following each harvest due to logistic problems, and many are dependent on subsidies from NGOs for assistance in obtaining fingerlings, paying for transportation costs and for other inputs (Meyer, 2001). There are several private farms, national fish culture stations and universities in Honduras that specialize in tilapia reproduction and distribution of sex-reversed fingerlings (Green and Engle, 2000). The quality and sales price of fingerlings available in Honduras is variable (Aceituno et al., 1997; Triminio et al., 2004).

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

Small-scale fish farms are widely distributed throughout Honduras. Honduras is primarily mountainous with limited and difficult access to communities via many roads that are useable for only part of each year. This makes the acquisition of tilapia seed difficult for small-scale fish farmers in many parts of the country. Many small-scale tilapia farmers have been dependent on NGOs that assist them in aquaculture activities to obtain and transport their seed.

The development of fingerling production capabilities in additional areas of the country will provide a greater degree of independence for the farmers to obtain seed locally, contributing to the elimination of this NGO subsidy. Our efforts should contribute substantially to making tilapia culture more viable and sustainable in rural areas of Honduras and in other parts of Latin America.

In addition, production of all-male tilapia fingerlings can be a very profitable business for local fish farmers (Engle, 1986; Popma and Green, 1990). The level of profitability in fingerling production is dependent on utilizing appropriate technologies and the proper management of fish and other inputs (= costs). There is a documented unsatisfied demand for tilapia seed in many areas of Honduras (Triminio, 2001; Aquaculture CRSP, 2002). These activities will provide locally generated information useful for producing appropriate materials in Spanish for implementation of training courses in Honduras and other countries in Latin America.

Quantified Anticipated Benefits

Direct Target Groups of the Study Will Be:

- A minimum of 50 individuals (fish farmers and extension agents from NGOs and government agencies) from Honduras, El Salvador, Nicaragua, Guatemala, Haiti and Dominican Republic will receive technical training in the areas of brood-stock selection and management, tilapia reproduction, as well as the production and distribution of fingerlings.
- Over 50 producers will receive profiles of the costs for Nile and red tilapia fingerling production in Honduras. This information will be disseminated through a variety of media and formats, including formal presentations at scientific meetings, teaching and training events held in Zamorano and posting on the inter-net.
- A training manual and Power Point presentations on techniques for tilapia brood-stock selection, reproduction and fingerling production, will be developed in Spanish for utilization in CRSP training events.

Indirect Target Groups Will Be:

- We expect that more than 2000 small-scale tilapia farmers will benefit by having better quality tilapia fingerlings available locally in Honduras and in the other countries included for training of farmers and extension agents.
- A minimum of 50 individuals from several Latin American countries will be trained in the selection and management of tilapia brood-stock, and in techniques for tilapia reproduction and fingerling production.

These activities will enhance Zamorano´s and Auburn's institutional understanding of the status of small-scale tilapia culture in the Latin American region and our abilities to propose and undertake effective development projects with aquaculture components.

The information and training materials derived from this study will be useful for aquaculture development activities in other countries of Central and South America.

Activity Plan

Activity 1: Training Events

We plan to organize and carry out two training events in Central America and one on the island of Hispañola during year-two of WP11. The two four-day long training events in Central America will held on the Zamorano campus to facilitate implementing a program that includes both theoretical and practical aspects ("learning by doing") of fish reproduction and fingerling production. Training programs will present alternative methods of producing tilapia fingerlings including mixed-sex, hand-sorted, and sex-reversed. For small producers in remote areas where production objectives primarily focus on food security, mixed-sex fingerlings may be a viable strategy. Hand-sexing may present employment and business opportunities for some talented individuals. Sex-reversal technology is legal and generally accepted by consumers in Central America and is widely used by commercial fingerling producers. We will invite farmers and extension agents from Honduras, El Salvador, Guatemala and Nicaragua to participate in these events.

We will organize and hold a four-day training event in the Dominican Republic. The organization of the event (site and specific dates) and identification of potential participants will be jointly done by the CRSP Co-PIs from Honduras, with assistance from the following individuals and agencies:

- Blas Santos, Latin American Programs Coordinator, Kellogg Foundation, D. R.
- José Espaillat, Agricultural Education Officer, Ministry of Education, D. R.
- Father Jack Charles, Vincent Foundation, Haiti
- Ariel Azael, Quisqueya University, Haiti
- José Cordero, Zamorano Alumni Association, D.R.
- Pilar Ramírez, Agricultural Consultant, D.R.

Each participant in each of the training events will be evaluated to determine the effectiveness of our technology transfer his/her demonstrated level of learning by answering a series of questions at the start and finish of each event.

These training events will provide us with opportunities to continue our inventory of NGOs promoting tilapia culture in the region. All of the information from the inventory will be stored in an electronic database for analysis, easy access and use, by all interested parties. This database will form part of the www.acuacultura.org website.

Through these training events we will provide technical expertise to extension agents and farmers assisted by Action Against Hunger, the National Autonomous University of Honduras (UNAH), the Center for Studies of the Ocean and Aquaculture at the San Carlos University in Guatemala, the National Institute for Professional Formation in Honduras (INFOP) and other regional organizations and agencies.

<u>Activity 2: Investigation</u>

Adult mixed-sex Nile and red tilapia (Jamaica strain) will be stocked at 2 fish/m^2 (1 male% to 3 female& fish) into four 200 m^2 ponds, two ponds for each tilapia genetic line, for the production of tilapia fry (Popma and Green, 1990). Prior to stocking, all fish will be rested for a minimum of 15 days. The adult fish will have an average weight of approximately 150 g. A similar biomass of female fish will be stocked into each experimental pond.

Partial harvests of fry will be made on alternate days beginning at their first appearance in each pond. All harvested fry will be subsequently sex-reversed in 7 m^3 concrete tanks at 2000/m^3 at the Zamorano Aquaculture Station. All experimental ponds will be drained and adult fish harvested at 30 culture-days. Sac-fry and eggs will be collected from the mouths of any incubating females for evaluation at final pond harvesting.

The entire experimental protocol will be repeated following a 15-day rest period for the fish. The adult fish will be resorted and stocked into the 200 m^2 ponds for an additional 30-day reproduction period and the fry sex-reversed in tanks. The experimental design would include two treatments (Nile and red tilapia) and a total of four repetitions.

All fry will be sex-reversed using a dose of 60 mg of methyl-testosterone/Kg of feed containing 32% crude protein. The feed prepared with MT will be offered to the fry ad libitum, four times each day, during 28 days.

The production budget will include the value for each direct cost (water, fish, feeds and fertilizers, labor, equipment, use of vehicles) and indirect costs (administrative costs, depreciation of ponds and equipment).

Data from these trials will be used to develop and compare production budgets for the sale and distribution of sex-reversed Nile and red tilapia fingerlings under local conditions. The number of fry harvested from the experimental ponds stocked with Nile and red tilapia adults and hormone treated fingerlings harvested from the 7 m^3 tanks will be compared using an ANOVA.

Many small and medium-scale fish farmers in Honduras and neighboring countries continue to have difficulty obtaining tilapia fingerlings for stocking ponds. Often fingerlings produced in Honduras are of poor quality ($\geq$ 5% females, non-uniform in size and color). The production budgets will be developed by calculating all direct (inputs such as water, feeds and fertilizers, brood-stock, labor, and others) and indirect costs (depreciation of equipment and installations, others).

Activity 3: Developing Training Materials

Training materials on brood-stock selection and management, tilapia reproduction and fry production techniques will be organized and published in Spanish. The materials will include a written training manual and Power Point files. These materials will be used in our training events to instruct selected fish farmers and NGO and government extension agents in the fundamentals of tilapia brood-stock selection and management (Lutz, 2001), techniques for reproduction, and fingerling production and distribution. Information for developing these materials will be derived largely from CRSP supported research and extension materials (Egna and Boyd, 1997), and from work done at Zamorano. Original photographs and line drawings will be included from Zamorano archives or produced as needed, to illustrate the manual.

A draft copy of the manual will be reviewed and evaluated by a professional editor from the Zamorano communications department. The technical content of the manual will be evaluated and critiqued by fish culture specialists, fellow CRSP researchers, and by a panel of potential end-users including extension agents and fish farmers.

Regional and Global Integration

We plan a formal integration of this activity with the overall region. The work will be jointly conducted by Zamorano personnel (faculty, staff and students). In-country personnel (D. Meyer and S. Meyer) will assist in organizing all training sessions and in development of training materials. The training events will contribute to strengthening our contacts in countries where we have previously interacted and provide opportunities for new contacts in countries that have not had prior relations with the CRSP.

Schedule

The fish farmer and extension agent training events in Central America and on the island of Hispanola will be held during the period 1 August 2005 and 30 June 2006.

The investigation of Nile and red tilapia fry production costs will be done between March and September 2005.

The preparation and publishing of a manual on tilapia brood-stock selection, reproduction and fingerling production will be completed by December 2005.

Report Submission

As was done for the previous work plan, copies of the research finding and recommendations were made available at training sessions. A report spanning the two years of this activity is planned to be submitted to the Aquaculture CRSP by 30 June 2006.

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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

Seedstock Development and Availability 2 (11.5SDA2)/Experiment/Kenya

Investigators

Charles C. Ngugi	HC Principal Investigator	Department of Fisheries, Moi University
Bethuel Omolo	HC Principal Investigator	Fisheries Department, Government of Kenya
Chris Langdon	US Principal Investigator	Department of Fisheries and Wildlife, Oregon State University
James Bowman	US Principal Investigator	Department of Fisheries and Wildlife, Oregon State University

Objectives

1) Assess management strategies that may contribute to improved growth and survival of African catfish (*Clarias gariepinus*) juveniles in the hatchery and in ponds. Strategies that may be investigated include:
    - Varying the duration of the rearing phase and assess its effect on growth and survival of juvenile catfish reared in indoor aquaria and in hapas suspended in static ponds;
    - Offering live, freeze-dried, and formulated feeds to hatchery-reared larvae in different sequences, rather than offering them a single feed throughout rearing period; and
    - Varying the stocking densities of catfish juveniles reared in the hatchery and hapas suspended in static ponds.
2) Assess the economics of raising catfish juveniles offered live feeds or live feeds followed by dry feeds.

Significance

Efforts to grow fish in ponds in Kenya began in early 1920s. However, rural fish farming dates back to 1940s when farmers in Central and Western Kenya started to construct fish ponds to culture Nile tilapia. In spite of several decades of fish culture, Kenya's aquaculture has not come far and remains a young industry, practiced mainly on a small scale using tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) and producing only about 1,000 metric tons annually.

Clarias gariepinus is probably the most widely distributed fish in Africa (Skelton, 1993). It can endure very harsh conditions by using its accessory air breathing organ, is highly omnivorous, and grows quickly. The fish has high potential for aquaculture in this region. Research into the culture potential and the artificial propagation of catfish is reported to have begun in the 1970's. (DeKimpe and Micha, 1974; Hogendoorn, 1984).

Clarias are farmed in polyculture with Nile tilapia to control unwanted reproduction. They are also grown in monoculture as food fish. More recently, *Clarias* culture has gained in importance as a way of producing bait fish for the Lake Victoria Nile perch industry. The traditional supply of fingerlings is wild-caught fingerlings from Lake Victoria itself, but this supply is intermittent and seems to be related to the extent of water hyacinth rafts drifting in near-shore areas, with *Clarias* being numerous under the water hyacinth. Fishers sometimes use small-mesh beach seines and mosquito nets to catch fingerlings for bait, but beach seining is highly destructive of the spawning habitats of native cichlids and is illegal. Fishing with mosquito nets has also recently been banned by the government. Fishers find themselves in a difficult situation because they need the bait at an affordable price to be able to continue fishing.

Poorer fishermen have resorted to long-line fishing and their favorite bait is *Clarias* fingerlings. Desired bait size varies widely, ranging from 5 to 20 grams per fish. A quick survey of fishermen gave hugely varied numbers of fishers and number of hooks per fisher, but it seems that fishers bait from 100 to 1000 hooks per boat per day, and the demand in Kenya is between 5,000 and 50,000 *Clarias* fingerlings per day. At 300 fishing days per year, this equates to an annual demand of between 1.5 and 15 million fingerlings. At a reported selling price of 2 to 3 KSh per fingerling and an estimated production cost of about 0.1 KSh per fingerling (Veverica, Personal Communication, 2001), farm-based production could be a highly profitable business for fish farmers. Although recent thesis work by one of our students estimated that fingerling production costs would be just under KShs1.00, his investigation still suggested that producers could realize returns of 400% or more on their investments.

The African catfish, *Clarias gariepinus*, is endemic to the Lake Victoria region. It is popular with the communities living around the lake and a lot consider its taste as excellent. Like many other indigenous fish species tried in aquaculture, lack of adequate seed is a drawback to production of catfish. Spawning of *Clarias* is not a major problem, but sufficient quantities of fingerlings cannot be produced due to very low survival to fingerling size. Spawning success as reported by Karen Veverica (Personal Communication) has been quite good, but survival to fingerling size is variable, ranging from 1 to 50% in ponds, with a survival rate of 25% from egg to 5-gram fingerling considered good.

Work on increasing the survival of *Clarias* fry to the fingerling stage was begun under CRSP sponsorship through a series of experiments conducted at Moi University, Eldoret, Kenya, in 2002 and 2003 (Ngugi et al., 2004). That work included studies on suitable first feeds and appropriate stocking densities for *Clarias* fry nursed indoors in 30-L glass aquaria, as well as studies on appropriate stocking densities and varied amounts of cover provided for fry reared in hapas in outdoor ponds. Results from the indoor studies suggested that near-pure cultures of rotifers produced in fertilized earthen ponds might successfully be used, in place of *Artemia*, to rear *Clarias* fry in tanks—although the growth of fry fed *Artemia* was better than that of fry fed rotifers, the difference was not statistically significant. On the other hand, survival of fry fed rotifers was higher than that of fry fed *Artemia*, although not significantly so. The density study indicated that the best stocking density, in terms of both growth and survival, was about 10 fry/L (tested densities were 5, 10, 20, and 30 fry/L). In the outdoor pond/hapa studies, better survival and growth of fry were achieved when the hapas were 100% covered with grasses, as opposed to when 0-, 30-, or 60-% covered. Of the hapa stocking densities tested (100, 200, and 400 fry/m^2), the best appeared to be about 100 fry/m^2. Because this density was the lowest among those tested, further work on lower hapa stocking densities appears to be in order.

Results from the above experiments suggest that additional work should be done to further increase survivals in both indoor systems and outdoor ponds and hapas. Accordingly, this proposed activity will address how length of the nursery rearing phase, stocking densities, alternative feeding strategies, and factors such as predation and cannibalism affect the growth and survival of *C. gariepinus* fry.

Quantified Anticipated Benefits

Research results will be applied to the production of *C. gariepinus* fingerlings on both government and private farms. The catfish culture section of the aquaculture handbook developed under an earlier CRSP project can be updated with new information and technologies coming out of this research. New information or technologies resulting from this research will be transferred to extension workers and farmers producing *Clarias* fingerlings through training courses conducted both at the FD and MU fish farms and at the farms of advanced *Clarias* producers, where it is anticipated that applicable sections of the aquaculture handbook will be used in the training. Increased supplies of *Clarias* fingerlings will provide Lake Victoria Nile perch fishers with a reliable source of bait. Fishing pressure on immature *Clarias* in Lake Victoria will be reduced. Reduction in beach seining will reduce habitat destruction on native fishes in Lake Victoria. Net income to fishermen may increase if baitfish is more available and if costs are kept down through competition among bait producers. A steady supply of *Clarias* fingerlings will also help producers in areas where *Clarias* is gaining popularity as a cultured food fish. Increases in fish production realized through all these avenues will contribute to human health and welfare in the region.

Collaboration on this project will also strengthen the institutions involved—the MU Fisheries Department and the Kenya Fisheries Department—as well as further strengthen the linkage between them. Graduate training provided for FD personnel will further build on the strengths of that Department.

Research Design

Location of Work: These experiments will be conducted at the Moi University Fish Farm (Chepkoilel Campus) in Eldoret, Kenya, and/or at Sagana Fish Farm, Sagana, Kenya.

Pond and Hatchery Facilities: Two experiments will be conducted, either at Sagana Fish Farm, Sagana, Kenya, or at the Moi University Fish Farm, Eldoret, Kenya.

Moi University:
- 12 ponds measuring 10 x 10 m (100 m^2); and
- 24 30-litre indoor aquaria.

Sagana:
- 12 ponds measuring 10 x 15 m (150 m^2); and
- 12 30-litre indoor aquaria.

Test Species: *Clarias gariepinus* (African catfish)

Culture Period: 21 to 42 days, depending on the experiment (see details below)

Stocking Rates:

Hatchery Aquaria: 10 larvae/L, or best rate from new density study, if conducted.

Hapas in Ponds: This is the variable for the proposed hapa stocking density study—proposed densities for that study are 25, 50, 100, and 200 larvae/m^2.

Nutrient Inputs:

Hatchery Aquaria: none (fish are fed, but aquarium water is not enriched)

Ponds:
- Initial fertilization with urea and DAP at 10 kg N and 4 kg P/ha plus chicken or cow manure at 500 kg/ha 2 days prior to stocking.
- Repeat at ½ initial dose on days 7, 14, and 21.
- Feed twice daily beginning on day 4:10kg/ha/d, formulated diet (36% protein). Feed will be put at the grass/water margin.

Water Management: Ponds and hatchery aquaria will be managed as static water systems, with water added only to compensate for evaporation or seepage, or, in the case of aquaria, for losses incurred during cleaning. Accumulated debris will be siphoned from the bottom of each aquarium twice daily, and 60% of the aquarium water in each tank will be exchanged every fifth day, immediately following water quality sampling.

Sampling Schedule:

Water Quality Sampling—Hatchery: Dissolved oxygen, temperature, and pH in each tank will be measured daily, at 0900 and 1600 hours. TAN, and Nitrite-N in each tank will be determined every fifth day.

Water Quality Sampling—Ponds: Dissolved oxygen and temperature will be determined every fifth day at 0700 and 1700 hours, at three depths (5, 25, and 50 cm) and three points in each pond (for hapas and pond experiments). Samples for pH, TAN, and nitrite-N will be collected every fifth day. Secchi disk visibility will be measured at the open end of the pond every five days. Temperature will also be monitored hourly using temperature data loggers.

Fish Sampling: Fish will be sampled weekly for growth (weight and length) and survival. Juveniles will be weighed to the nearest milligram and total length taken to the nearest millimeter.

Potential Experiments: Two graduate (MSc) students will be supported under a companion activity under this Work Plan ("Aquaculture Training for Kenyan Extension Workers, Fish Farmers, and University Students"). Under guidance from the project PIs, and subject to practical considerations, at least two of the following experiments will be selected by those students, to be conducted at Moi University or Sagana Fish Farm.

Experiment A: Variation of the Length of the Hatchery Rearing Phase

Duration: Variable.

Treatments: Three replications each of larvae reared for periods of 21, 28, 35, and 42 days.

Feeding: Six times daily, at two-hour intervals. Our best available feeding strategy from previous experiments will be used, with only the length of the hatchery rearing phase being varied.

Experiment B: Sequential Feeding of Different Live Feeds to Hatchery-Reared Larvae

Duration: 28 days.

Treatments: Three replications each of larvae offered *Artemia*, Rotifers, Freeze-dried *Cyclops* or formulated feeds in different sequences, with possible combinations as follows:

- *Artemia* for 14 days, followed by rotifers for 14 days;
- *Artemia* for 7 days, followed by rotifers for 21 days;
- *Artemia* for 7 days, followed by rotifers for 14 days, followed by *Cyclops* for 7 days;
- Rotifers for 28 days;
- Rotifers for 14 days, followed by *Artemia* for 14 days;
- Rotifers for 7 days, followed by *Artemia* for 21 days;
- Other variations of the numbers of days on each possible feed; and
- Other combinations including formulated feeds as a feed alternative.

Feeding: six times daily, at two-hour intervals.

Experiment C: Variation of Stocking Densities for Larvae Reared in Hapas in Ponds

Duration: 28 to 42 days.

Treatments: Three replications each of larvae stocked into hapas at densities of 25, 50, 100, and 200/m^2.

Feeding: formulated diet (36% protein).

Experiment D: Assessment of the Effects of Rearing Period on Growth and Survival of Juvenile Catfish Reared in Indoor Aquaria and in Hapas Suspended in Ponds

Duration: Variable.

Treatments: Three replications each of larvae reared for periods of 5, 10, and 15 days after hatching.

Feeding: Six times daily, at two-hour intervals. Our best available feeding strategy from previous experiments will be used, with only the length of the hatchery rearing phase being varied.

Statistical Design, Null Hypotheses, Statistical Analyses: The results of these experiments will be analyzed using one way ANOVA and regression techniques. Null hypotheses are that stocking density, cannibalism, feeds, and length of grow-out period do not have significant effects on survival, growth, or production of fry or on net returns from fingerlings produced and sold.

Regional Integration

By encouraging collaboration among officials of the FD and MU, this activity addresses one of the Regional Plan's overall goals, "To encourage and support cooperation, communication, and technology transfer among research and extension institutions in the region." By providing support to faculty of the MU Department of Fisheries and officers of the FD in guiding and mentoring graduate students conducting aquaculture research, the proposed research addresses the additional goal "To encourage and support the strengthening of research and extension institutions throughout the region." Finally, this proposed work addresses a key aspect of the overall CRSP objectives for the current grant, which is "the development of culture systems for local and native species" (New Aquaculture Systems/New Species Theme).

Schedule

- Students will be selected in September 2004.
- Course work will begin as soon as possible after selection, but not later than 1 January 2005.
- Students will begin writing their research proposals as soon as they begin their coursework.
- Pond and/or hatchery research will be conducted between August 2004 and February 2006.
- Theses will be ready for presentation to committees by 31 April 2006.
- The status of all activities will regularly be submitted in quarterly reports.
- Final reports on this experimental work will be submitted to the CRSP by 30 May 2006.

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

Seedstock Development and Availability 3 (11.5SDA3)/Experiment/Mexico

Investigators

Carl B. Schreck	US Principal Investigator	Oregon State University
Guillermo R. Giannico	US Principal Investigator	Oregon State University
Wilfrido M. Contreras Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco
Mario Fernández-Perez	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco
Ulises Hernández-Vidal	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco

Objectives

1) Obtain broodstock from an F5 generation of wild and introduced stocks of Nile tilapia based on traditional genetic selection.
2) Provide tilapia fry farms in Central America with quality broodstock.

Significance

In Latin America, broodstock and seed supply have been identified as one of the major constraints to production increases. In the 2001 expert panel meeting organized by the Aquaculture CRSP, inadequate availability and quality of fry (and broodstock) were listed as a researchable priority. In southeastern Mexico, tilapia broodstock and fry quality are particularly poor. In the region, tilapia culture has been the principal aquacultural activity since the early 1970s. Unfortunately, the loss of the introduced lineages, the lack of effective genetic selection programs, and poor management decisions have created disappointment and uncertainty regarding tilapia culture. Thanks to CRSP funding (Eleventh Work Plan), we have effectively obtained an F3 generation selected based on the combination of total length and condition factor. Comparisons made against the old broodstock group used in the government owned farm, a major seed producer in the region, showed that our broodstock produced an average 2.4 times more fry than the old line. Malformations are not present in the fingerlings produced, and survival and growth rates have been improved and we are currently raising an F4 generation from the F3 broodstock. Despite these encouraging results, the selection of at least an F5 generation is needed to ensure good quality broodstock. It has been reported that the selection of a fourth and fifth generation allows for the fixation of traits in a given tilapia population thus decreasing heritability values (Tave and Smitherman, 1980; Sanchez and Ponce, 1988; Tave, 1993, 1996).

The selective breeding program supported by the CRSP from 2001 to 2003 was initiated using 220 females and 110 males obtained from a batch of fish purchased from Egypt by the state government. A second line is currently being selected from wild animals. We have identified a stock of wild Nile tilapia in the Usumacinta River that shows several advantageous phenotypic traits (small head, small tail, large body, and uniform color). For the first year of work, we were able to combine the efforts of the CRSP project and another supported by the National Council for Science and Technology (CONA-CyT-Mexico). This action allowed us to work at the Mariano Matamoros Hatchery using 200, 1,000, and 2,000 m^2 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.

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

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

Quantified Anticipated Benefits

Traditional genetic selection will generate a group of fish that will be used as broodstock capable of producing larger numbers of high quality fry than currently available. Fry and broodstock will be distributed to farmers. One workshop on tilapia reproduction and line selection will be conducted at UJAT every year.

Research Design

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

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

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

Group 1. Control group (old line from the Mariano Matamoros Farm)
Group 2. F4 Egypt line (CRSP project)
Group 3. F4 wild line (CRSP project)

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

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

Line Selection: Ten percent of the fish from the growth performance phase of the Egypt and wild lines will be randomly collected. Fry will be divided in three groups using weight as the selection variable: 1) Fry which are 33% above the median value, 2) fry which are 33% around the median value, and 3) fry which are 33% below the median value. Group 1 will be reserved for follow-up studies, and group 2 will be used for line selection. All fish in group 3 will be discarded. From group 2 (of each line), 14,000 fish will be stocked in 1,000 m^2 earthen ponds (14 fish per m^2) and grown-out for 2 months. At this time, 35% of the population (4,000 fish) that have the highest length will be selected and placed in 1,000 m^2 earthen ponds. After six months of growth, 35% (1,400) of the fish with the highest length will be selected to produce the F5 generation. Males will be selected based on highest condition factor, and females will be selected based on highest length.

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

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

Universidad Juárez Autónoma de Tabasco (DACA): Two spawning concrete ponds (50 m^2), two grow-out ponds (50 m^2), 20 net cages (1 m^3) for grow-out.

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

Stocking Rate for Spawning: 3 females to 1 male, per 3 m^2.

Stocking Rate for Grow-Out: 14 fish per m^2 (first 2 months); 4 fish per m^2 (2 months); 20 fish per m^2.

Culture Period: 9 months.

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

Nutrient Inputs: None.

Water Management: 10% water exchange per day. Temperature, pH and dissolved oxygen, will be measured daily. Nitrates, nitrites and ammonia will be measured once a week, during the duration of trials.

Sampling: The following variables will be sampled:
Initial weight and length
Survival
Final weight
Final length

The following values will be estimated:
Growth rate
Condition factor
Food conversion factor

Statistical Methods and Hypotheses: H_o: Broodstock selected from the F4 generation will not have higher growth or fry production than those from the wild or the control groups. H_A: Broodstock selected from the F4 generation will have higher growth or fry production than those from the wild or the control groups. To determine differences in growth performance and fry production, ANOVA will be performed using weight, length, and mean number of fry per female as the dependent variables.

Regional and Global Integration

If the results of the proposed study are successful, we will exchange information with Daniel Meyer at the Pan-American Agricultural School. We have already discussed our intentions to exchange information and facilitate technology transfer with other institutions.

Schedule

Data collection, November 2004–June 2006; Final technical report, 30 June 2006.

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Development of Aquaculture Techniques for the Indigenous Species of Southern Mexico, *Centropomus undecimalis*: Sex Determination and Differentiation and Effects of Temperature

Seedstock Development and Availability 4 (11.5SDA4)/Experiment/Mexico

Investigators

Reynaldo Patiño	US Principal Investigator	Texas Tech University (TTU)
Kevin Pope	US Principal Investigator	Texas Tech University (TTU)
Wilfrido M. Contreras Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco (UJAT)
Gabriel Márquez Couturier	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco (UJAT)
Salomón Páramo-Delgadillo	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco (UJAT)
Ulises Hernández-Vidal	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco (UJAT)

Goal

To develop culture techniques for common snook in southern Mexico.

Objectives

1) Determine the timing and morphological pattern of gonadal sex differentiation in common snook fry and juveniles.
2) Determine the effect of early treatment with low or high water temperature or sex steroid on sex ratios and growth rate of common snook.

Significance

Artisanal fisheries based on the capture of wild populations are the primary current source of fish for the food market in the southern region of Mexico. However, because of its geographic and hydrological features, this region also has been considered one of the most promising areas in Mexico for the development of aquaculture. Although native fishes are deeply embedded in the culture of the region and constitute important food staples for its people, to date most aquacultural programs have relied primarily on non-native species such as tilapias and carps. These exotic species have escaped the confines of aquacultural farms and are now reported to have invaded biologically sensitive areas such as The Pantanos de Centla Biosphere Reserve (Tabasco), the most important wetland system in southeastern Mexico. The impact of these exotics on the ecological viability of the area remains largely unexplored but is likely to be considerable. This proposed work is based on the premise that the development of aquaculture of indigenous species is preferable for the region in the context of both market acceptability and ecological compatibility.

Species of "robalo," or snook, are among the most important indigenous fish species along the Mexican coastline of the Gulf of Mexico. Among the species of snook, the robalo blanco, or common snook (*Centropomus undecimalis*) are caught in relatively greater numbers and enjoy a high market value (Anonymous, 2002). During the last few years, the average annual catch for common snook has been approximately 5,000 tons in Mexico, and 900 tons for the state of Tabasco (Anonymous, 2002). However, there is an overall national trend for diminishing catch volumes despite occasional and short-lived local increases, a situation that has led to concerns for the health of the regional snook fisheries and to calls for improved management practices (Anonymous, 2002). The natural range of common snook extends from North Carolina to Brazil (Muller et al., 2001), and therefore the status of natural snook populations is also of international concern. In places such as Florida (USA), common snook were until recently considered a "species of special concern" for which commercial harvest was banned, and strict management regulations are currently in place for its recreational fisheries (Anonymous, 2001).

Reproductive Biology of Common Snook and Current Status of Its Aquaculture:

Knowledge of the reproductive biology of common snook is limited. Histological observations of the gonads of fish collected from the field are consistent with the concept that common snook are protandric hermaphrodites. Namely, they appear to first develop as males and thereafter reverse into females. In Florida, all fish younger than 1-2 years (depending on site of collection) seem to be males, an even sex ratio is observed at 5-7 years, and most fish older than 12-15 years seem to be females (Taylor et al., 2000). Interestingly, the available data (Taylor et al., 2000) also indicates that within the same age class, females are larger than males. This size differential is particularly pronounced in younger fish, 1-2 years old, where the fork length of females is 60-70 percent longer than that of males (Tables 2 and 3 in Taylor et al., 2000). The spawning season for common snook runs from spring through early fall, depending on the geographical location, during which they spawn multiple times (Peters et al. 1998; Taylor et al. 1998). Although it is sometimes assumed that sex reversal in common snook occurs after their first spawning (Muller et al., 2001), there is histological evidence suggesting that males may retain their gender through consecutive spawning seasons (Grier and Taylor, 1998). Additional studies are clearly needed to obtain a better knowledge of the reproductive biology of common snook. Further, most studies of common snook reproduction have focused on Florida populations and research with populations in other geographical locations is necessary to determine the general applicability of the results obtained.

Techniques for aquaculture production of snook are at present not fully developed. Snook broodstock has been difficult to maintain in captivity and thus the few available hatchery-spawning programs have relied on wild-caught fish. Wild-caught broodstock are either immediately processed upon capture to obtain gametes for in vitro fertilization, or they are brought to the hatchery where they are promptly injected with hormones to induce spawning (Anonymous, 2001). Fingerlings for stocking purposes have been successfully produced using this technique.

Rationale for Proposed Research:

The finding that female snook are larger than males of the same age class, especially in the younger fish (see preceding discussion), suggests that females have an intrinsically faster growth rate than males. This finding has obvious and important relevance for the development of aquacultural techniques for snook. For example, sex determination in most fishes is readily manipulated by exogenous administration of sex steroids early during gonadal development (Schreck, 1972; Hunter and Donaldson 1983; Contreras, 2001). Also, in many species including those with strong genetic determinants of sex, sex ratios can be manipulated by changes in water temperature (Strüssmann and Patiño, 1995, 1999; Patiño et al., 1996). Control of sex ratios by environmental techniques such as water temperature has the advantage that they are not associated with real or perceived concerns for the use of steroids ("endocrine disruptors") in aquaculture. Therefore, this proposed project is designed to determine if sex ratios of common snook can be changed in favor of females by manipulation of water temperature or application of exogenous sex steroids (estrogen), and if growth rate is associated with gender. To our knowledge, the effects of temperature on sex determination of naturally hermaphroditic teleost species has not been examined, but in other protandric teleosts the exogenous administration of estrogens generally results in female formation (Guigen et al., 1993; Condeca and Canario, 1999; Lee et al., 2000).

The development of an aquaculture industry for common snook would benefit the Gulf Coast region of Mexico for various important reasons. It would be consistent with regional plans for the development of aquaculture as a source of income and of food fish. It would also be consistent with the common-sense premise that the use of indigenous species for aquaculture has a much lower probability of causing ecological damage compared to exotics such as tilapias or carp (see preceding discussion). Finally, it would provide relief from the intense fishing pressure currently being exerted on wild snook populations.

Anticipated Benefits and Impact Indicators

Incorporation of native species, such as common snook, is needed for the further development of Mexican aquaculture. This study will focus on the evaluation of techniques that may result in enhanced growth rates of farmed common snook by manipulating sex ratios to favor the formation of faster-growing females. Culture techniques for native species will benefit fish farmers in Southern Mexico and Central America by promoting aquacultural diversification while avoiding the potentially harmful ecological effects associated with the widespread use of exotic species. If the proposed protocols provide positive results, farm trials will be set up at Ejido Rio Playa and workshops will be conducted to train farmers, students and technicians. One graduate student and one undergraduate student will be directly involved in the project, and it is anticipated that many more undergraduate students will also participate during the course of the study. At least one publication in a refereed scientific journal is expected from the results of this proposed study.

In addition, this project would strengthen ties between TTU and UJAT and foster current efforts to establish collaborative research projects between the two institutions. In support of these efforts, R. Patiño and K. Pope traveled to Villahermosa, Tabasco, in 2001 to participate in a symposium on reproductive physiology and early life history of fishes. The symposium was organized under the auspices of UJAT.

Experimental Design

All experiments will be conducted at UJAT with the assistance of a graduate student from TTU and personnel from UJAT.

Experiment 1: Timing and Morphological Pattern of Gonadal Sex Differentiation in Common Snook

Methods: Snook fry will be obtained by collection of ripe wild broodstock (during spawning season in the summer) and in vitro fertilization of eggs. Fish will be fed Artemia nauplii initially, and trained to accept artificial feed afterwards.

Laboratory and Pond Facility: UJAT; fish will be initially maintained in 20–40-l tanks and transferred to circular 140 l tanks as the fish grow in size.

Culture Period: One year.

Stocking Rate: Initially 100 fry/tank. After treatment or as needed due to growth, juveniles will be transferred to recirculating systems with 18-140 l tanks for grow out. If fish require more time for grow-out, fish will be transfer to 1m^3 mosquito mesh hapas.

Test Species: Common snook (*Centropomus undecimalis*).

Nutrient Inputs: none

Water Management: Water will be maintained at 27.5º C, 25% water exchange once a week. Water pH, DO, and temperature will be monitored daily.

Sampling Schedule: Samples for histological inspection of the developing gonads will be collected on the day of hatching, at bi-weekly intervals during the first 2 months post-hatch, and monthly thereafter for a total period of one year. Sample size will be 10 fish per sampling time. Criteria for gonadal differentiation will be as described by Patiño and Takashima (1995).

Statistical Methods and Hypothesis: This is a purely descriptive study whose purpose is to provide the basis for deciding the timing of the experimental manipulations in Experiment 2. Thus, statistical analysis will not be necessary. All fish are anticipated to form an immature testis within the first year of hatching, most likely within the first several weeks or months.

Schedule of Performance: Data collection, November 2004–June 2006; Final technical report, 30 June 2006.

Experiment 2: Effect of Early Treatment With Low or High Water Temperature or Sex Steroid on the Sex Ratio and Growth Rate of Common Snook

Methods 2a: Manipulation of water temperature. Juvenile snook cease feeding at 14º C (Howells et al., 1990) and in their natural habitat they are found in temperatures as high as 40º C (Peters et al., 1998). Thus, the experimental temperatures in this study will be within the reported tolerance limits and include the following: 17.5, 22.5, 27.5, 32.5 and 37.5º C. The time of onset and the duration of the treatments will depend on the results of Experiment 1. The duration of the treatment will cover the normal period of testicular differentiation, and is expected to last about 4 weeks (see Strüssmann and Patiño, 1995, 1999).

Methods 2b: Administration of estradiol-17β. Oral administration of estradiol-17β will be via bioencapsulation using *Artemia nauplii*. Again, the timing of the treatments will depend on the results from Experiment 1.

Treatments: Experiment 2b consists of four treatments:

- fry fed bioencapsulated E2 for 7 days from onset of treatment (timing to be determined)
- fry fed bioencapsulated E2 for 14 days from onset of treatment
- fry fed bioencapsulated E2 for 21 days from onset of treatment
- fry fed bioencapsulated E2 for 28 days from onset of treatment
- Control treatment will be fed control food from onset of treatment

Laboratory and Pond Facility: UJAT; 5 recirculating systems with 12 20 l tanks each; 3 recirculating systems with 18-140 l tanks for grow out; 30 0.5m^3 hapas and 30 1.0 m^3 hapas.

Culture Period: One year.

Stocking Rate: Initially 100 fry/tank. After treatment or as needed due to growth, juveniles will be transferred to recirculating systems with 19-140 l tanks for grow out. If fish require more time for grow-out, fish will be transfer to 1m^3 mosquito mesh hapas.

Test Species: Common snook (*Centropomus undecimalis*).

Nutrient Inputs: None.

Water Management: Except for the temperature experiment (2a), in which temperature will vary during the treatment period, water will normally be maintained at 27.5º C with 25% water exchange once a week. Temperature, pH and dissolved oxygen, will be measured daily. Nitrates, nitrites and ammonia will be measured once a week, during the duration of trials.

Sampling Schedule: Ten samples will be collected for histological examination of the gonads prior to temperature or steroid treatment. Each treatment will be triplicated, and five samples will be collected from each replicate per treatment (15 total per treatment) at the end of each treatment. Also, at the end of the one-year grow-out period, all remaining fish (up to 300 per treatment) will be collected, measured for fork length and weight, and their sex determined by gross examination or, if necessary, histological examination. At sampling, fish will be killed with an overdose of anesthetic (MS-222).

Statistical Methods and Hypotheses: H_o2a: Changes in water temperature will not affect sex ratios or growth rates of the treated fish. H_o2b: Treatment with E2 will not affect sex ratios or growth rates of the treated fish. The efficacy of the treatments to change sex ratios will be tested comparing percentage of females between treatments with a Chi-squared test. Effects on final length and weight will be determined by 2-way ANOVA (treatment x sex), and differences in treatment means will be established by Duncan's multiple range tests. If necessary, data will be transformed to achieve homogeneity of variances.

Regional Integration

In Central America there are several farmers interested in the culture of *Centropomus undecimalis* but have no training on sex inversion. We plan to share our experiences with hatchery managers and workers and potentially establish experimental trials in those farms by implementing thesis projects with undergraduate students. We have already communicated with the Honduras Aquaculture CRSP research team concerning our intentions to exchange information and for potential technology transfer. Our target population will be small-scale farmers. We will promote aquacultural activities using native species and sex inversion. We have met with hatchery managers from Tabasco and Chiapas and they have expressed their interest in collaborating with UJAT in this enterprise.

Schedule: Data collection, November 2004–June 2006; Final technical report, 30 June 2006.

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Effects of Native Peruvian feedstuffs on Growth and Health of *Colossoma* and *Piaractus*

Fish Nutrition and Feed Technology 1 (11.5FNF1)/Study/Peru

Investigators

Rebecca Lochmann	US Co-Principal Investigator	University of Arkansas at Pine Bluff
Christopher C. Kohler	US Project Director and Principal Investigator	Southern Illinois University at Carbondale
Susan T. Kohler	US Co-Principal Investigator	Southern Illinois University at Carbondale
William Camargo	US Project Coordinator	Southern Illinois University at Carbondale
Salvador Tello	Host Country Project Leader	Institute for the Investigation of the Peruvian Amazon
Fernando Alcántara	Host Country Principal Investigator	Institute for the Investigation of the Peruvian Amazon
Palmira Padilla P.	Host Country Co-Principal Investigator	Institute for the Investigation of the Peruvian Amazon
Rosa Ismiño	Host Country Co-Principal Investigator	Institute for the Investigation of the Peruvian Amazon

Objectives

1) Assess the effects of native Amazonian feedstuffs on the health of *Colossoma* in a feeding trial.
2) Assess the effects of native Amazonian feedstuffs on the growth, survival and health of *Piaractus* in a feeding trial.

Significance

The aquaculture potential of native Peruvian species such as *Colossoma* spp., *Piaractus* spp., and their hybrids needs further study. Nutrition has a major impact on fish growth, health and overall performance and fish feeds are usually one of the main costs of fish culture. Currently, no uniform fish diets for Characids available in the Amazon region (Cantelmo et al., 1986; Ferraz de Lima and Castagnolli, 1989).

Characids can be reared successfully on relatively low-protein diets (Carneiro, 1981; Hernandez et al., 1992). Their natural diets are high in plant products (Kubitzki and Ziburski, 1993; Araujo-Lima and Goulding, 1997), making them ideal candidates for testing native plant feedstuffs in formulated diets. The seasonal availability of different plants is known, but the nutritional quality of these plants has not been addressed in detail. Recent unpublished digestibility data from Kohler et al. and Lochmann et al. indicates that several common Amazonian plants (yucca, plantain, and pijuayo) have potential as feedstuffs in prepared diets - especially pijuayo. A small-scale feeding trial conducted previously with pijuayo in place of corn in the diet also indicated the value of this feedstuff for *Piaractus* broodstock (Lochmann, 2002). Additional comparative studies on young, rapidly growing fish are needed on native plant feedstuffs to fully assess their potential. Aside from standard production data (growth, survival, feed conversion efficiency) the health impact of diets with these novel feedstuffs has not been evaluated. In this study, we will conduct a suite of health assays on *Colossoma* in a feeding trial conducted at SIU Carbondale by Kohler et al. in Fall 2004. In addition, we will conduct a feeding trial on juvenile *Piaractus* in 2005 to assess production and health of fish fed diets with yucca, plantain or pijuayo (identical diets to those used in the Colossoma feeding trial at SIU Carbondale). The combined results of these studies will provide a robust indication of the utility of different native plant feedstuffs for prepared Characid diets.

Quantified Anticipated Benefits

The development of practical diets using native plant feedstuffs will enhance the sustainability of

Characid aquaculture in the Amazon region be reducing dependence on non-native or more expensive feedstuffs, which should lower overall feed cost. Increased use of local resources will provide an ecologically sound method of improving production efficiency. Fish constitute a major portion of the protein intake in the Amazon region, and the provision of consistent plentiful supplies of desirable species should improve the quality of life for many residents.

This study in combination with the companion studies of Kohler et al. addresses CRSP goals of improving culture techniques for indigenous Amazonian species through the development of less expensive and better utilized feeds. Increased availability of cultured fish should enhance human nutrition and health. In addition, increased aquaculture production may relieve pressure on dwindling natural stocks of desirable foodfish species. In this study we will quantify the following:

- Number of diet formulations containing native plants that have a beneficial effect on health of *Colossoma.*
- Number of diet formulations containing native plants that have a beneficial effect on production and health of *Piaractus*.

Research Design and Activity Plan

Objective 1. Assess the Health Impact of Native Amazonian Plant Products as Feedstuffs for Small-Scale Production of *Colossoma*

A feeding trial is being conducted at SIU Carbondale to evaluate the feasibility of utilizing three native Amazonian plant products (plantain, *Musa paradisiacal;* yucca, *Manihot sculenta;* and pijuayo, *Bactris gasipaes)* to culture *Colossoma macropomum* in recirculating systems. Details of the trial are given in the WP 11.5 proposal submitted by Kohler et al. At harvest standard production data will be collected by Kohler et al. In addition, personnel from UAPB will collect blood samples to determine health indices of fish from this trial. The analyses will include lysozyme, alternative complement activity, hematocrit, hemoglobin, and MHCH. Before final weights are obtained, fish will be lightly anaesthetized with tricaine methanesulfonate (MS-222 at 20 mg l^{-1}) and approximately 0.5 ml of blood will be drawn into heparinized syringes and centrifuged (3500 x *g* for 10 min) to separate the blood fractions. Hematocrit and hemoglobin (Hb) content (Hb cyanide method, Houston, 1990) will be measured. Mean corpuscular hemoglobin concentration (MCHC) of individual fish will be calculated based on the formula: MCHC = Hb concentration/hematocrit fraction. For the lysozyme assay, 50 µl of fish plasma will added to each well in a 96-well plate. One hundred and fifty µl of suspended *Micrococcus lysodeikticus* (0.4mg/ml in phosphate buffer) will be mixed with the fish plasma in each well, and the reduction in the absorbance reading at 450 nm will be taken every 10 seconds for 5 min using the basic kinetic protocol of the microplate reader with SoftMax Pro 4.3 (Molecular Devices, Sunnyvale, CA). One unit of lysozyme activity is defined as a reduction in absorbance of 0.001 per minute (1milli OD/min). Approximately 25 µl of additional fish plasma will be used for a hemolytic assay of alternative complement activity. The hemolytic activity effected by the alternative complement pathway will be measured using washed rabbit red blood cells as target cells in the presence of EGTA and Mg^{2+}, as described by Tort et al. (1996).

Health data will be analyzed by one-way analysis of variance (ANOVA). Appropriate transformations will be made where necessary. The appropriate post-hoc tests will be employed when significant differences among treatment means are found. The accepted level of significance will be 0.05.

Objective 2: Assess the Growth, Survival, Feed Conversion Efficiency and Health Impact of Native Amazonian Plant Products as Feedstuffs for Small-Scale Production of *Piaractus*

The methodology for this feeding trial will follow that of objective 1 as closely as possible with only minor adjustments anticipated for species or system differences.

Null Hypothesis for Feeding Trials: There is no difference in growth performance or health effects in *C. macropomum* fed or *P. brachypomu*s fed diets containing different native plants.

Regional and Global Integration

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

Schedule

Preparation for initial feeding and digestibility trials will begin 1 October 2004. All trials will be completed by 30 April 2006.

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Nutrition and Nutrient Utilization in Native Peruvian Fishes

Fish Nutrition and Feed Technology 2 (11.5FNF2)/Study/Peru

Investigators

Christopher C. Kohler	US Project Director and Principal Investigator	Southern Illinois University at Carbondale
Susan T. Kohler	US Co-Principal Investigator	Southern Illinois University at Carbondale
William Camargo	US Project Coordinator	Southern Illinois University at Carbondale
Salvador Tello	Host Country Project Leader	Institute for the Investigation of the Peruvian Amazon
Fernando Alcántara	HC Principal Investigator	Institute for the Investigation of the Peruvian Amazon
Palmira Padilla P.	HC Principal Investigator	Institute for the Investigation of the Peruvian Amazon
Rosa Ismiño	HC Co-Principal Investigator	Institute for the Investigation of the Peruvian Amazon
Rebecca Lochmann	US Co-Principal Investigator	University of Arkansas at Pine Bluff

Objectives

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

Significance

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

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

Culture techniques for native Peruvian fishes could be advanced considerably with new information on nutrient utilization in fishes fed diets with different compositions. Although some of the basic nutrient requirements are known for Characids (St-Paul, 1985; Hernandez et al., 1995; Fernandes et al., 2001), there is no information on the availability of nutrients from feedstuffs of local origin. Even when cost and convenience of local feedstuffs are attractive, there is no advantage to using them in fish diets if the nutrients they contain are largely unavailable. The primary method of determining bioavailability of nutrients from individual feedstuffs is to determine the digestibility coefficients of different nutrients in individual feedstuffs during feeding trials. Digestibility coefficients for many of the feedstuffs used in current Characid diets have been determined recently (Fernandes et al., 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 the Eleventh Work Plan, we conducted digestibility trials with *P. brachypomus* on 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 IIAP (PERU). Digestibility coefficients were determined by using an indirect method, involving chromic oxide (Cr2O3) 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 to be employed 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 will be conducted in Work Plan 11.5 with *Colossoma macropomum* as well as a grow-out trial utilizing diets containing native plants as ingredients.

Quantified Anticipated Benefits

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

- Number of diet formulations containing native plants tested in growout trials with *Colossoma*.
- Number of diet formulations containing native plants tested for digestibility in *Colossoma*.

Research Design and Activity Plan

Objective 1: Assess the Feasibility of Utilizing Native Amazonian Plant Products for Small-Scale Sustainable Aquaculture Production of *Colossoma*

A nutrition study will be conducted to evaluate the feasibility of utilizing three native Amazonian plant products (plantain, *Musa paradisiacal*; yucca, *Manihot sculenta*; and pijuayo, *Bactris gasipaes*) to culture *Colossoma macropomum* in recirculating systems (Twelve 1000-gal. circular tanks connected to 3 biofilters and a drum filter with constant aeration) at SIUC and a control reference diet (catfish 32% protein). Each treatment (diet) will have three replicates (56 fish per tank and 168 fish per treatment). Fish will be fed 3% BW twice daily at 9 am and 4 pm. Fish will be sampled (50% of population) bi-weekly and weighed to adjust food rations. Growth will be monitored until achieving a commercial weight (by Amazon standards). Water quality parameters (pH, nitrite, nitrate, ammonia, CO2 and chlorides) will be monitored weekly and D.O. and temperature will be measured daily. At harvest, SGR, standing crop at harvest, condition (K), and feed conversion efficiency, survival (%) will be calculated. Data values will be analyzed by one-way analysis of variance (ANOVA). Appropriate transformations will be made where necessary. The appropriate post-hoc tests will be employed when significant differences among treatment means are found. The accepted level of significance will be 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 will be conducted to determine differences in digestibility of three native food sources (plantain, *Musa paradisiacal*; yucca, *Manihot sculenta*; and pijuayo, *Bactris gasipaes*) commonly fed to fish species cultured in the Peruvian Amazon. The three ingredients will be tested in *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 will be studied in combination with other ingredients in the assay diets. The reference diet will utilize menhaden fish meal because of the difficulty in acquiring anchovy fish meal in the US.

Digestibility trials will be conducted in 110-L tanks in a flow-through system at SIUC using the indirect method and with chromic oxide (1%) as the marker (Reilly and Lochmann, 2000). The assumption is 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 will be determined for each feedstuff. The reference diet will be similar in composition to those currently used for Characid fishes at IIAP (Peru). Water quality will be maintained by a flow rate of 36 L/min and a biofilter and bead filter. Temperature will be held at 27 +2^{o}C.

Five *C. macropomum* (~80 g) will be placed in each of nine randomly distributed 110-L tanks, each containing a digestibility chamber. Fish will be fed for a 6-day acclimation period followed by a 7-day fecal collection period. During the fecal collection period fish will be fed to apparent satiation once daily at 18:00 h and their feces collected 12 to 16 hours post-prandrial. Fecal samples will be stored frozen in airtight plastic bags. Feed and feces will be analyzed for dry matter (135 ^{o}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 will be analyzed by AOAC methods (1990). The digestibility of the nutrients will be determined by assessing the difference between the feed and fecal concentrations of the marker and the nutrient or energy. The percent nutrient digestibility will be estimated using the following formula:

Nutrient Digestion Coefficient = 100 - (100 % marker in the feed) (% nutrient in feces)/(% marker in feces) (% marker in feed)

The apparent digestibility coefficient (ADC) for a nutrient in each test ingredient will be calculated using the following expression:
ADC = 100/30 (dig. coeff. of test diet – 70/100 * dig. coeff. of reference diet).

Statistical Analysis (All Studies): Data of chromic oxide recovery will be 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*.

Regional and Global Integration

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

Schedule

Preparation for initial feeding and digestibility trials will begin 1 October 2004. All trials will be completed by 31 May 2006.

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Use of Phytochemicals as a New Method to Sex-Reverse Nile Tilapia and Tropical Garfish

Fish Nutrition and Feed Technology 3 (11.5FNF3)/Experiment/Mexico

Investigators

Konrad Dabrowski	US Lead Principal Investigator	Ohio State University
Jacques Rinchard	US Co-Principal Investigator	Ohio State University
Wilfrido M. Contreras Sánchez	HC Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Gabriel Márquez Couturier	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Ulises Hernandez-Vidal	HC Co-Principal Investigator	Universidad Juárez Autónoma de Tabasco, Mexico
Carlos A. Alvarez-Gonzales	HC Co-Principal Investigator	Universidad Juarez Autonoma de Tabasco, Mexico

Objectives

1) Evaluation of potential action of phytochemical extracts with a documented (in mammals) hormonal regulatory activity on sex differentiation in tilapia.
2) Development of starter diets and evaluation of potential action of purified phytochemicals on sex differentiation of tropical garfish (*Atractosteus tropicus*).

Significance

MT is known to be associated with gonad malformation problems when administered at high doses or over a prolonged time period. MT induces gonadal intersexuality and paradoxical feminization (Goudie et al., 1983; Solar et al., 1984; van den Hurk et al., 1989; Blasquez et al., 1995; Rinchard et al., 1999; Papoulias et al., 2000). Piferrer and Donaldson (1989) suggested that paradoxical feminization may be better explained by aromatization than inhibition of *in vivo* synthesis of androgens. Consequently, MT release and environmental concerns are a major reason behind a search for alternative, environment-friendly chemicals.

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

Besides the use of purified phytochemicals, we also propose the second approach, the use of plant extracts with known estrogenic characteristic of phytochemicals of interest. There is evidence that chemicals naturally occurring in plants could provide a useful source of masculinizing agents (Lohiya et al., 1999). Consequently, the detailed evaluation of such information is proposed in this Eleventh Work Plan. Several reports provide evidence that gastric intubation of aqueous extracts of *Hibiscus* *macranthus* and *Basella alba* into rat had anabolizing and virilizing effects (Moundipa et al., 1999) and may be used to boost low levels of testosterone in aging males. Maca (*Lepidium meyenii)* tuber meal has long been used as a remedy for human male's infertility in Peruvian rural communities (Quiros et al., 1996; Cicero et al., 2001; Gonzales et al., 2001a). Recently, oral administration of maca extracts was reported to enhance sexual behaviors in male mice and to decrease erectile dysfunction in male rats (Zheng et al., 2000). Cicero et al. (2001) also reported that the oral administration of pulverised maca root improved sexual performance of male rats. In rats, aqueous extracts of maca roots administered for 14 days increased weights of testis and epididymis, and enhanced spermatogenesis (Gonzales et al., 2001b). Testosterone and 11-keto testosterone play a role in sperm production and enhance male sexual characteristics. We hypothesize that maca and other plant extracts will have androgenic effects similar to testosterone/11 keto-T, that would potentially reverse females tilapia to males. Our laboratory in Columbus has extensive experience in using maca meal as feed intake enhancer in rainbow trout alevins and juveniles (Lee et al., 2004; Dabrowski et al., 2003) and this new aspect may add to a wide use of this plant material in aquaculture.

Large-scale introduction of tropical garfish in Southeastern Mexico will contribute to the recovery programs of wild populations and promote a different type of aquaculture; one based on sustainability of native species and also enable food security in the region. At present, all biological information on tropical garfish (Contreras et al., unpublished data) indicates that it will make a very good candidate for aquacultural purposes.

<u>Quantified Anticipated Benefits</u>

The use of phytochemicals as an alternative method to produce monosex populations of tilapia and garfish will address human and environmental safety issues. Fish offered to the consumer will not be treated with MT and producers may have an alternative method for producing all-male populations of tilapia based on natural products, which may not require FDA approval. The low cost of using phytochemicals or plant extracts should be also attractive alternative to producers. Moreover, several phytochemicals have proven antioxidant activity (Lee et al., 2005) (prevent oxidation of dietary lipids) and enhance immune resistance of fish.

We submit that a major undertaking in culture of tropical garfish in UJAT will provide additional impetus for aquaculture research and CRSP visibility in the local research community and appreciation by the local farmers/producers. The overarching problem in respect to both species, tilapia and garfish, is to produce monosex fish for stocking and intensive rearing purposes using environmentally-sound and acceptable methods. Using natural substances in that process will be a great benefit to both farmers and the general public. Some experiments may be performed on longnose garfish in OSU laboratory in Columbus and this will be the opportunity to gather new information on species not raised thus far in laboratory conditions. This would provide the opportunity for UJAT personnel to visit Columbus, collaborate and learn new techniques in diet formulation and analysis.

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

Location of work: The feeding experiments will be performed both at the Laboratory of Aquaculture, Universidad Juarez Autonoma de Tabasco, Mexico, and at the School of Natural Resources, The Ohio State University, Columbus, Ohio. The preparation of experimental diets and the HPLC analysis of phytochemicals will be carried out at OSU. The Aquaculture Laboratory at OSU has extensive experience in nutrition, reproduction, and sex reversal in tilapia (Mbahinzireki et al., 2001; Rinchard et al., 2002). We continue to work on phytochemicals in fish diets and their metabolism (Lee et al., 2001; 2004; Dabrowski et al., 2001). Over the years, several experiments in our laboratory involved the use of MT and monitoring concentrations of this chemical in fish tissues (Rinchard et al., 1999). Current studies on tilapia include monitoring quercetin, isoflavoinoids, and caffeic acid in tissues of tilapia (Rodriguez et al., 2005; Park et al., 2005).

Histological data generated from current research at UJAT indicates that tropical gars show undifferentiated gonads until day 39 post hatching, by day 54 gonads developing into ovaries are recognizable, and both sexes are differentiating by day 69 post hatching (Contreras, personal communication). This information clearly indicates that the process of gonadal differentiation is occurring between day 39 and 69. Literature descriptions suggest dimorphic growth in sex differentiated individuals of longnose garfish at the age of 1 year (Johnson and Noltie, 1997). OSU and UJAT laboratories will simultaneously perform baseline studies to determine sex differentiation in longnose garfish and in tropical garfish, respectively.

Methods:

Study 1. Evaluation of Potential Action of Phytochemicals Present in Plants With an Expected Hormonal Regulatory Activity on Sex Differentiation in Tilapia

Dietary formulations to be used in these experiments will be similar to those described in previous CRSP reports, i.e. casein-gelatin based diets with cellulose replaced on a weight-to-weight basis with plant extracts. Specifically, plants to be tested in the diets are: mate (*Ilex* sp.) (Filip et al., 2001), maca (*Lepidium meyenii*) (Quiros et al. ,1996; Lee et al., 2004; Li et al., 2001); aguaje (*Mauritia flexuosa*) (Alcantara, F., Iquitos, Peru, personal communication*), Hibiscus macranthus* and *Basella alba* (Moundipa et al., 1999). Preliminary experiments with maca meal in rainbow trout demonstrated significant effect on growth of fish at 15% dietary level (Lee et al., 2004). Therefore, we will use initially either 15% dietary substitution (maca and aguaje) or equivalent amount of extracts (see Lee et al., 2005 for details). Plant materials will be sequentially extracted with hexane, dichloromethane, ethyl acetate, methanol, and hot water. Methanol extract will be subjected to further fractionation in accordance to the procedure described by Olszewska and Wolbis (2002). In total, 6–8 diets will be formulated for each separate experiment. Activity guided fractionation will be used to isolate the fraction (or chemical) with most potent androgenic/aromatase inhibitor activity that induced masculinization of all female tilapia. Spironolactone has also shown some promise as a sex-reversal agent in our experiments in Mexico. We will expand this line of research, and diets supplemented with spironolactone (500 mg/kg) will be tested combined with phytochemical extracts.

Dietary treatments will include a negative and one positive (MT) control diets. Triplicate groups of all-female tilapia larvae after yolk absorption will be tested for 8-week duration. This period will include feeding diets with active ingredients (week 1–4) and continuation on control diet (week 5–8).

At the end of the feeding trial, growth performance will be evaluated in terms of individual body weight, survival, specific growth rate, and weight gain. Fish from each dietary treatment will be sampled for proximate body composition and phytochemicals analysis (Lee and Dabrowski, 2002a, b). Gonadal development and differentiation will be determined by histological analysis at week 4 or sex ratios will be evaluated by microscopic analysis of gonadal squashes (Guerrero and Shelton, 1974; Guigen et al., 1999; Lin et al., 1997) at the end of the experiment.

Study 2. Development of Starter Diets and Evaluation of Potential Action of Purified Phytochemicals on Sex Differentiation of Tropical Garfish (*Atractosteus tropicus*)

Experiments will be conducted in the laboratory at UJAT, Tabasco, Mexico. As a surrogate species, longnose gar (*Lepisosteus osseus*) from Ohio River or Lake Erie, Ohio, USA, can be used for some specific experiments with nutrient requirements. The OSU group has experience in working with longnose gar biochemistry (Moreau and Dabrowski, 2000). Facilities to be used in Columbus for larval garfish rearing were described in detail previously (Dabrowski et al., 2003; Lee et al., 2004).

An induced reproduction will be performed in May-August 2005, using matured females injected with carp pituitary or LHRHa (Sigma Chemical Co., St Louis, MO) (Contreras, W., personal communication). Fish will be then placed at a 1:3 female:male ratio in 2.5 m diameter spawning tanks (7 replicates). Fish will be provided with an artificial substrate and eggs are deposited within a few days. Embryos will be incubated in original spawning tanks (aeration, without flow), and hatched larvae (2–3 days at 26–28^{o}C) will remain in the tanks for 4–5 days until yolk sac absorption is completed. At this time, larvae will reach 10–12 mm in length (Contreras, W, personal communication) (18 mm; according to Mendoza et al., 2002) and they will be ready to be transferred to experimental rearing units.

Fist Feeding Experiment—Starter Diets:

At UJAT, 5 rearing recirculating systems will be used combining 12, 20 L tanks each. Fish will be stocked at 20 per L and rearing with live and/or formulated diets is projected for 30 days after initiation of feeding. Water for rearing units will be reciculated using bio-filter and 25% of the volume will be exchanged twice a week. Live brine shrimp *nauplii* will be used as a control diet. The formulated diets will consist of an experimental casein-based diet (particle size 200-355 µm, enriched with attractants (soluble fish protein concentrate and/or maca meal; Lee et al., 2004), a salmonid starter diet (BioDiet Starter 1, BioOregon, particle size 0-600 µm), and a larval marine fish diet (AgloNorse Larva Feed, EWOS, Norway, particle size 300-600 µm and 2 mm). In addition, two diets supplemented with genistein (500 mg/kg) or estradiol (E2) (20 mg/kg) will be used as a positive control. The rational for testing maca meal in garfish diets includes presence of sitosterol in this product (Li et al., 2001). The estrogenic effect of sitosterol has been reported in fish species, such as goldfish, rainbow trout and brown trout (see Lee et al. 2005 for references). The preferred diet formulation for E2 supplement is a semipurified diet, however, a preliminary test in Tabasco revealed that a casein/gelatin diet was not accepted by tropical garfish as the first food (Contreras, W., personal comm.). Therefore, as an alternative, we may use a fish meal-based diet as a basis in our project. A sub-sample (10 fish) from each tank will be counted and weighed after 15 and 30 days. This sub-sample will be used to estimate the average weight of individual fish in each tank. Tanks will be cleaned twice daily with a siphon to remove any dead fish, feces, and uneaten food.

Second Feeding Experiment—Feeding with Active Plant Extracts:

Weaning with marine (AgloNorse) and experimental diets should allow us to decide which diets will be used for tests of plant extract supplementss. A marine diet proved to be exceptionally successful in rearing common carp (and other cyprinids), Amazonian catfish (*Pseudoplatystoma fasciatum*) and lake sturgeon larvae from the first exogenous feeding (Froschauer, 2004; Dabrowski, personal observations). Therefore, it is highly probable that this diet will be also successful with garfish larvae. We will also adopt an alternative strategy where fish initially offered live *Artemia* will be transitioned to formulated diets at different ages (sizes). Initially, we will use either 15% dietary substitution (maca and aguaje) or equivalent amount of extract. Plant materials will be sequentially extracted with hexane, dichloromethane, ethyl acetate, methanol and hot water. Methanol and water extracts will be used in the present study. The positive control, a diet supplemented with estradiol (E2) at 20 mg/kg (Tzchori et al., 2004:; 70% feminization in eel) and genistein (500 mg/kg) will be used. This E2 level is higher than what was used previously by Contreras et al. (personal comm.) (1.5 mg E2/kg live *Artemia*).

Alternatively, in these experiments fish will be fed live *Artemia nauplii* for 14 days and then switched to either the experimental (particle size 355-500 µm) or marine (particle size 600 µm) larval diets for an additional 15 days. Triplicate tanks will be used for each treatment. In addition, fish in treatment 1 will be switched from feeding with live *Artemia* to the marine larval diet after 3 days. Fish in treatment 2 will be switched from feeding with live *Artemia* to the marine larval diet after 6 days. Total experimental feeding for all treatments will be 30 days. The remaining fish will be weighed individually.

At the end of the feeding trial, growth performance will be evaluated in terms of individual body weight, survival, specific growth rate and weight gain as described earlier. Fish from each dietary treatment will be sampled for proximal body analysis (Lee and Dabrowski, 2002a, b) and histological analysis. Gonadal development and differentiation will be determined by histological analysis at 30 days (Rinchard et al., 2002). In the case of insufficient differentiation of the gonads at this time fish from several dietary treatments will be reared until the size of 10-12 cm using commercial diets. We will then attempt to evaluate sex ratio by identification criteria described in larger Lepisosteidae (Ferrara and Irwin, 2001), microscopic analysis of gonadal squashes (Guerrero and Shelton, 1974; Guigen et al., 1999) or by histological analysis (Lin et al., 1997) at the end of the experiment.

Statistical Analysis:

Specific growth rate (SGR) will be calculated for each tank as SGR = {(ln (final weight)-ln(initial weight) x 100)/(number of days fed)} and expressed as a percentage day^{-1}. Daily survival rates will be calculated as the percentage of live larvae. Percentages will be arc sine transformed prior to statistical analysis. Feeding treatments will be compared by one-way analysis of variance (ANOVA) and Tukey's pairwise comparisons using Minitab 11.0 statistical software (Zar, 1999) or followed by a comparison of means using Scheffe's F test. The Chi-square test will be used to determine alterations in sex ratios. Differences will be considered significant at $P < 0.05$. Analyses will be performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

Regional Integration

Among the States of Mexico, Tabasco is considered to have the greatest potential for both intensive and extensive aquaculture development. Moreover, fish consumption constitutes an important part of the rural lifestyle in the State of Tabasco. Therefore, the research efforts being proposed are logical initial steps towards developing sustainable aquaculture of native species in the region, in particular tropical garfish. The research will benefit the entire region by providing pertinent information on masculinization of tilapia or feminization of garfish using natural phytochemicals.

Schedule

Study 1. Evaluation of Potential Action of Phytochemicals Present in Plants on Sex Differentiation in Tilapia

- January–March 2005, formulation and preparation of the experimental diets;
- April–June 2005, production of all-female and all male tilapia population;
- July–August 2005, first set of feeding experiments and sampling;
- August 2005, measurement of phytochemicals in tilapia tissue;
- September 2005, second set of experiments; and
- October–December 2005, data analysis and preparation of reports and publications.

Study 2. Development of Starter Diets and Evaluation of Potential Action of Purified Phytochemicals on Sex Differentiation of Tropical Garfish (*Atractosteus tropicus*)

- August–September 2005, preparation of the experimental diets and acceptance tests with garfish larvae;
- October–December 2005, feeding experiments and sampling;
- January 2006, analysis of gonadal histology and sex reversal ratio,
- January–February 2006, second set of experiments; and
- March–June 2006, data analysis and preparation of reports and publications.

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Water Quality Monitoring and Identification of Pollution Sources Leading Towards Classification of Bivalve Growing Waters

Aquaculture and Human Health Impacts 1 (11.5AHH1)/Study/Mexico

Investigators

Maria Haws	US Principal Investigator	University of Hawaii, Hilo
John Supan	US Principal Investigator	Louisiana State University
Eladio Gaxiola	HC Principal Investigator	Universidad Autonoma de Sinaloa, Mexico
Emilio Ochoa	HC Principal Investigator	Ecocostas, Ecuador
Pamela Rubinoff	Collaborator	University of Rhode Island

Objectives

This investigation will be one component of the Working Group's efforts to develop capacity, resources and guidelines to classify bivalve growing waters in coastal Mexico to support the shellfish industry to more fully realize it's potential. Classification of shellfish growing waters according to Mexican and US requirements will increase the ability of producers to grow safe shellfish, increase consumer acceptability and provide a competitive edge to coastal communities employing sanitation guidelines. This will be particularly beneficial to women's groups who are growing and selling shellfish. It also provides economic incentives for coastal communities and local governments to take steps to assure the environmental integrity of coastal waters. This work also links to concurrent efforts by some of the members of the CRSP Pacific Mexico Working Group promoting community clean-up efforts, latrine programs, reduction of industrial waste (including shrimp farm wastes) and other initiatives designed to reduce health impacts and environmental degradation.

Bivalve shellfish such as clams, oysters, and cockles readily accumulate pathogens, heavy metals and other contaminants such as pesticides in their tissues through their filter feeding action. To prevent shellfish consumption causing human diseases such as cholera, *vibrio* or other waterborne illnesses, the all US States require shellfish growing waters to be monitored and classified according to their current and probable levels of contamination. This is regulated by the Inter-State Shellfish Sanitation Committee (ISSC). John Supan was selected as an advisor for this work because of his long history working with the ISSC, particularly on international issues.

Waters assessed to be suitably free of real and possible contamination such that consumption of shellfish grown in these waters is determined to be safe are classified as "approved" growing waters. Growing areas can also be classified as "conditional", "restricted" or "prohibited" (ISSC, 2003). Mexico has similar guidelines but these are not considered adequate to allow for importation to the US and local implementation is patchy. Currently only two shellfish growing areas in Mexico are certified to export to the US; both are locate in Ensenada, Baja California. This demonstrates that complying with US guidelines is feasible for some Mexican producers. However, working to satisfy Mexican health standards is the first step toward improving shellfish safety and quality and will go a long ways to improving sales and revenues for producers and the health of consumers. Aside from monitoring and classification of growing areas, post-harvest processes such as handling and shipping are also covered by guidelines in both nations, but these issues are primarily being addressed in non-CRSP efforts.

The Pacific Mexican States such as Sonora, Sinaloa and Nayarit are also in a good position to export shellfish to the US if water quality can be certified to be safe for shellfish production. The Pacific oyster (*Crassostrea gigas)* is widely produced with most of the seed being supplied by two large US companies. There is also growing interest in the indigenous oyster species, *Crassostrea corteziensis* which is the traditional culture species in the state of Nayarit and is being re-introduced to Sinaloa. This species has good export potential, particularly as a raw, half shell product given its desirable characteristics such as: better taste than *C. gigas* when raw; rapid growth; similarity in appearance to *C. viriginca* (the most popular half shell oyster for North America); and its attractive appearance, including the light rosy shell color found in some specimens. Several other bivalve species such as blood cockles (*Anadara* spp.), clams (multiple genera) and pen shells (*Atrina maura*) can also be cultured and exported to ethnic markets in the US. These species are also popular in Mexico, with the pen shells being of particularly high value ($15.00/pound). There is also great potential to market locally to the high-end tourist market (e.g. Mazatlan, Acalpulco, Los Cabos, Tijuana) if these consumers can be assured of the safety of the product through labeling or certification.

This effort will take the first steps toward determining which areas of Agua Brava Bay in the state of Nayarit and the Bahia Santa Maria Bay in Sinaloa should be targeted for monitoring and classification efforts in the future. Agua Brava Bay is the most heavily used oyster culture area in Nayarit. Bahia Santa Maria is the current focus of a bay management conservation plan that includes elements of developing alternative livelihoods. Several of the organizations and individuals involved in this work have plans in action to enable several fisher women's groups in BSM to begin growing bivalves (oysters and pen shells). This work will therefore help current producers in Nayarit evaluate their present growing areas, select new growing areas, make management decisions and guide new producers in BSM.

Classification of bivalve growing waters requires a minimum of one year of intensive monitoring and is costly. To avoid wasting resources on current or potential growing areas which experience sources of pollution or other conditions such that obtaining approved status within the classification system is improbable, this work will broadly survey the Agua Brava Bay and BSM to determine which areas should be the focus areas for the more intensive monitoring efforts in the future. Additionally, the findings will inform the oyster producers so that they can make decisions regarding mitigation efforts for current activities that may be affecting the safety of their shellfish and help identify areas that might replace contaminated growing areas or be good sights for future expansion.

Specifically, this initiative will:

1) Team international specialists and HC specialists to develop monitoring methods based on Mexican and US requirements for classification of waters.
2) Work jointly to begin preliminary monitoring of the major bivalve growing area in Pacific Mexico as a prelude to more focused monitoring for the purpose of classifying specific growing areas.
3) Identify point source pollution in two bays.
4) Estimate volumes and seasonal variation in waste water sources affecting the bays.
5) Provide additional information to supplement previous modeling of the hydrodynamics of the systems.
6) Monitor total and fecal coliform levels.
7) Analyze and interpret results to determine where further monitoring for purposes of classification would be most feasible.
8) Identify sources of pollution or other activities which may impede classification in some growing areas and develop strategies for mitigation.
9) Conduct awareness raising and outreach to officials, community members and scientists to work towards long-term, institutionalized efforts to classify waters.
10) Update the biography of the work conducted on hydrodynamics and toxicology of the bays and surrounding areas.
11) Identify precise areas of both bays that would then be subject to more intense monitoring for the purpose of classifying growing waters in future efforts.
12) Provide a data base, maps and initial zoning of the two bays as the basis for future classification of growing waters.
13) Producers, community leaders and government officials will be involved through inclusion in planning and work sessions as a means of awareness raising and to promote engagement in the policy and technical aspects of this work.

Significance

The group of institutions and researchers collaborating on this work has targeted the development of native species and species which have low technology requirements as priorities for the Pacific region. Traditionally, aquaculture development and associated research and extension in Pacific Mexico have been dominated by shrimp culture. Although still the largest aquaculture sector, the series of epidemics over the last ten years, rising operational costs and lower prices have put many shrimp farms out of business and lowered the profitability of others. This has particularly impacted the smaller cooperative shrimp farms owned by ejidos. Shrimp culture in Sinaloa and Sonora is also limited to some degree by cold winter temperatures that permit only one or two crops per year; adding more cold-hardy species to the production scheme would allow for year round income. Additionally, as the coastal population grows and as the traditional economic mainstay of fishing also declines, new alternatives are needed. Diversification of aquaculture holds great potential for new producers and to allow shrimp farmers and cooperatives to diversify away from shrimp.

Of all the potential species for diversification, bivalves are among the best candidates for efforts since bivalve culture already exists on a small scale, technology requirements are low, environmental impacts are few and the extensive Pacific coast tidal flats and bays represent prime grow out grounds. Many of these areas are still relatively environmentally pristine. Over 50 potential bivalve species have been identified as candidates for culture and many of these are high value, both on the local and export markets. Aside from the potential for export to the US, Pacific Mexico is a primary tourist destination for North Americans and Europeans. While many of the potential culture species would be considered rather exotic by traditional North American standards, many of the same species are popular throughout Latin America, Europe and Asia. As shellfish stocks in these areas decline or growing areas become more contaminated, the relatively pristine waters of Pacific Mexico and intact shellfish stocks represent a largely unexploited potential for growers in Mexico if environmental quality can be preserved and preventative guidelines put in place.

This work will focus on growing areas where women's groups already produce *C. gigas* and *C. corteziensis* and or have shown interest in producing other high value species such as blood cockles and pen shells. Classification of growing waters or identification of areas that could be acceptable growing areas according to Mexican and US standard not only increases the real safety of consuming shellfish products, but also enhances the image of shellfish and increases the likelihood of consumer acceptance. The health of producers, local consumers and consumers outside the local areas will be protected if safer shellfish can be produced. Household food security and health of coastal communities will be improved if shellfish production and prices are increased. Additionally other benefits may accrue if the awareness of sanitation and water quality issues among the households and communities involved in shellfish production is increased. Economic incentives for improving community sanitation through initiatives such as garbage collection, latrine building and increased sanitation for food producing and serving facilities will benefit the communities and their visitors in other ways.

<u>Quantified Anticipated Benefits</u>

Target Groups: Aquaculture extension workers and researchers in Pacific Mexico; key private sector representatives; participants from selected CRSP projects in the LAC region; the Bahia Santa Maria Management Committee; Women's Cooperatives of BSM; Women's oyster culture cooperatives of Nayarit; Women's oyster culture cooperative of Puerto Peñasco; Conservation International; Universidad Autonoma de Sinaloa (UAS, Culiacan and Mazatlan Campuses); ISA; Ecocostas; CESASIN; SENASICA, SAGARPA, CONAPROF, and the fisher women's community groups of BSM. Two Host Country students will be involved in this work and will benefit from the hands-on learning experience.

Quantifiable Benefits:

1) Development of monitoring programs and increases in skills associated with water quality monitoring; Identification and/or classification of safe shellfish areas;
2) Development of monitoring schemes; findings that may lead to classification of growing areas; safer shellfish;
3) Possible increases in production and revenues;
4) Diversification of aquaculture; and
5) Alternatives to shrimp culture.

Activity Plan
Monitoring and identification of pollution sources and impacting activities will be conducted in two major bay systems, Agua Brava in Nayarit and Bahia Santa Maria in Sinaloa. Some previous hydrodynamics modeling has been done for both systems with the BSM system being the more intensively studied. Monitoring stations will be established in each system in areas selected either because shellfish culture is already being conducted or because they are pre-identified as the most likely areas for expansion of bivalve culture. In the case of BSM, the sites will also be selected with the accessibility to the fisher women's community groups which are in the planning stages of developing bivalve culture taken into consideration. Monitoring will include sampling and analysis for total and fecal coliform as well as precise identification of sources of pollution which will be monitored for seasonal variability. Additional information will be collected on the hydrodynamics of each system to supplement previous work. This work will be conducted for a period of one year.

The US National Shellfish Sanitation Program's (NSSP) Model Ordinance will be used as a guide for conducting site surveys and training as well as the Mexican national and state sanitary standards. The Model Ordinance, promulgated by the Interstate Shellfish Sanitation Conference (ISSC), addresses required procedures and standards for compliance by state and foreign shellfish sanitation programs for the interstate shipping and/or importing of molluscan shellfish in the US Specific chapters dealing with the classification of growing waters will be translated from English to Spanish.

The final products will include GIS maps identifying sources of pollution and activities potentially affecting the ability to classify areas as approved growing areas. Estimates will also be derived as to the flushing times and patterns of each bay system. Based on the findings, each system will zoned according to criteria indicating the probability of being a good candidate area for sanitary shellfish production. A final report will be prepared and distributed to the various user groups. This work will also form the basis for outreach activities with governmental and stakeholder groups to work towards implementation of formal programs for classification of growing waters in each system, as well as programs targeted towards mitigating point source and non-point source pollution (Investigation 3). Guillermo Rodriguez, Department Head and Nicolas Castaneda, Fisheries Scientist, School of Marine Sciences, UAS-Mazatlan Campus will conduct the monitoring and data analysis.

Regional and Global Integration
This work is a continuation of the Tenth Work Plan to build extension capacity and resolve issues related to human health and aquaculture. This work is linked to conservation and sustainable development initiatives undertaken by URI, UHH, UAS, Conservation International and CESASIN in the Pacific states of Sonora, Sinaloa and Nayarit. This includes the Santa Maria Bay management initiative, management of the Marismas Nacionales (wetland systems in Nayarit), development of best management practices for shrimp culture (URI/UHH/CI/David and Lucille Packard Foundation) and aquaculture development efforts by the above mentioned institutions. Additionally, this work will contribute to a series of international extension training efforts to be undertaken for a five year period by the USAID/EGAT-sponsored SUCCESS project (Sustainable Coastal Communities and Ecosystems) in other CRSP regions such as Nicaragua, Ecuador and East Africa.

Schedule
Start Date: 1 June 2005.
End Date: 30 June 2006.

Task	Month												
	J	J	A	S	O	N	D	J	F	M	A	M	J
Monitoring of bay systems	X	X	X	X	X	X	X	X	X	X	X	X	
On-going development of maps			X	X	X	X	X	X	X	X	X	X	
Final report													X

Outreach and Planning for Implementation of Bivalve Growing Areas Classification and Related Sanitation Action Items

Aquaculture and Human Health Impacts 2 (11.5AHH2)/Activity/Mexico

Investigators

Maria Haws	US Principal Investigator	University of Hawaii, Hilo
John Supan	US Principal Investigator	Louisiana State University
Eladio Gaxiola	HC Principal Investigator	Universidad Autonoma de Sinaloa, Mexico
Emilio Ochoa	HC Principal Investigator	Ecocostas, Ecuador
Pamela Rubinoff	Collaborator	University of Rhode Island

Objectives

This work addresses Goals 3. 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 proceed and as consumer awareness of the 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 2 and will be aimed at disseminating the findings of the 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 2 as well as the outcomes of the Tenth Work Plan. 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 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. A major participant in this work will be SENASICA (National Service for Agricultural Product Health, Safety and Quality), the agency primarily responsible for agricultural sanitation issues.

Specifically, this work will:

1) Raise awareness among key institutional and community stakeholders about the major issues associated with aquaculture sanitation.
2) Educate stakeholders about the technical and legal requirements for safe production of bivalves.
3) Disseminate findings, outcomes, lessons learned and strategies from previous work and Investigation 2 to the authorities and key stakeholders.
4) 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.
5) Develop and accept an implementation plan for the above mentioned topics.
6) Two students from UAS (to be identified) will participate in this work.
7) Producers, community leaders and government officials will be involved through inclusion in planning and work sessions as a means of awareness raising and to promote engagement in the policy and technical aspects of this work.

Significance

The Tenth Work Plan findings and previous work conducted by the Sinaloa Working Group has found that poor water quality and lack of community sanitation programs are among the greatest impediments to further aquaculture development and represent the greatest threats to human health and welfare in many ways. While many areas of the coast are still relatively pristine, threats to environmental quality and human health are intensifying as coastal populations increase and human activities alter the coast line. The ability to culture, harvest, process, handle, ship and market safe aquaculture products is severely affected by contaminated waters and generally impairs hygiene in coastal communities. The Working Group has a long history of experience working with these issues and in the case of the communities in BSM, has developed community-based programs such as community garbage collection, latrine building programs, environmental education and related efforts that are making positive impacts on sanitation. This work needs to be replicated, but also expanded into new areas such as shellfish sanitation at all levels.

The ability to develop legal and technical procedures to cultivate safe, high quality shellfish will be a major contribution to expansion of this industry and towards assuring the health of consumers. Additionally, other measures to mitigate impacts from point source and non-point pollution that affects water quality and aquaculture product safety through a variety of causal connection are sorely needed in coastal areas. Reaching out to policy makers, regulatory agencies and key stakeholders is critical if these measures are to be implemented. This work will provide a means of connecting researchers, regulators and users to work towards implementation of these much needed actions to improve water quality. Improvement of water quality will not only help strengthen benefits from shellfish culture, but will have multiple benefits for communities and aquaculture producers.

Quantifiable Anticipated Benefits

A major partner in this work is SENASICA which is based within the Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA) and which works closely with CONAPROF (National Consultative Committee for Standardization), the body overseeing standards, product certification and verification of standards. Other target groups for this work include: aquaculture extension workers and researchers in Pacific Mexico; key private sector representatives; participants from selected CRSP projects in the LAC region; the Bahia Santa Maria Management Committee; Women's Cooperatives of BSM; Women's oyster culture cooperatives of Nayarit; Women's oyster culture cooperative of Puerto Peñasco; Conservation International; Universidad Autonoma de Sinaloa (UAS, Culiacan, and Mazatlan Campuses); ISA; Ecocostas; CESASIN; and the fisher women's community groups of BSM. Two Host Country students will be involved in this work and will benefit from the hands-on learning experience.

Quantifiable Benefits:

1) Development of monitoring programs and increases in skills associated with water quality monitoring; identification and/or classification of safe shellfish areas;
2) Development of monitoring schemes; findings that may lead to classification of growing areas;
3) Safer shellfish;
4) Possible increases in production and revenues;
5) Diversification of aquaculture; and
6) Alternatives to shrimp culture.

Activity Plan

A series of three meetings will be held with the participants to include scientists, regulators, policy makers, aquaculture producers and community members from the target communities in BSM and Agua Brava. The first meeting will be for the purpose of informing stakeholders about the activities of the working group related to shellfish culture, shellfish sanitation and general community sanitation as related to aquaculture development. The first steps towards developing a program for shellfish sanitation will be the topic of work at this meeting. A second meeting will be held to update the group as to project findings to date and to continue work on the implementation plan for shellfish sanitation. The third meeting will be for the purpose of presenting final results from Investigation 2 and the other ongoing activities and finalizing the implementation plan for shellfish sanitation for the two bay systems.

Regional and Global Integration

This work is a continuation of the Tenth Work Plan to build extension capacity and is linked to conservation and sustainable development initiatives undertaken by URI, UHH, UAS, Conservation International, and CESASIN in the Pacific states of Sonora, Sinaloa and Nayarit. This includes the Santa Maria Bay management initiative, management of the Marismas Nacionales (wetland systems in Nayarit), development of best management practices for shrimp culture (David and Lucille Packard Foundation) and aquaculture development efforts by the above mentioned institutions. Additionally, this work will contribute information and lessons learned to the series of international extension training efforts to be undertaken for a five year period by the USAID/EGAT-sponsored SUCCESS project (Sustainable Coastal Communities and Ecosystems) in other CRSP regions such as Nicaragua, Ecuador, and East Africa.

Schedule

Starting Date: 1 June 2005.
End Date: 30 June 2006.

Task	Month											
	J	J	A	S	O	N	D	J	F	M	A	M	J
Meeting 1, development of outline for implementation plan for shellfish sanitation		X											
Meeting 2, further work on implementation plan and update on research findings							X						
Meeting 3, presentation of final outcomes from study and finalization of implementation plan											X		
Publication of implementation plan and distribution												X	X