Pond Dynamics/Aquaculture Collaborative Research Support Program

TENTH WORK PLAN

1 August 2001 through 30 April 2003

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This work plan describes a standardized set of experiments to be undertaken by the Collaborative Research Support Program in Pond Dynamics/Aquaculture during the period 1 August 2001 and 30 April 2003. Program activities are funded in part by Grant No. LAG-G-00-96-90015-00 from the United States Agency for International Development (USAID), Global Bureau, Office for Agriculture and Food Security. The authors' opinions expressed herein do not necessarily reflect the views of USAID.

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Contents of the Tenth Work Plan

The Tenth Work Plan of the Pond Dynamics/Aquaculture CRSP describes activities to be conducted by the CRSP from 1 July 2001 through 30 April 2003 under United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00. Previous activities under this grant are described in the Eighth Work Plan, which covered the period from 1 August 1996 to 31 July 1998, and in the Ninth Work Plan, which covered the period from 1 August 1998 to 31 July 2001.

The goal of the current grant is to provide a basis for improving the sustainability of aquaculture production systems. Under the grant framework, research in production systems is organized into three research areas—Production Optimization, Environmental Effects, and Social and Economic Aspects—which are further subdivided into specific research themes. Descriptions of the research themes can be found on pages 140 to 141. The table on pages 4 and 5 illustrates the distribution of Tenth Work Plan investigations among the three research areas, and further among research themes.

Work under the Tenth Work Plan will be implemented at sites in Mexico, Honduras, Peru, Kenya, the Philippines, and Thailand. As for companion site and regional outreach, Tenth Work Plan projects also involve collaboration with researchers and institutions in El Salvador, Nicaragua, Brazil, South Africa, Bangladesh, Cambodia, Laos, Nepal, and Vietnam. The table on pages 6 and 7 illustrates the distribution of Tenth Work Plan investigations among these host countries.

Development of the Work Plan

The CRSPs current grant was originally proposed as a five-year program, spanning from 1 August 1996 through 31 July 2001. After the award of a two-year extension by USAID, the grant completion date is now 31 July 2003. The program was successful in demonstrating to USAID the value of extending the period of the current grant term by two years to allow the planned objectives of the research portfolio to be fully met; the Tenth Work Plan is the means to achieve that end. Because of a series of annual budget cuts, the program had by necessity addressed grant objectives selectively in the Ninth Work Plan—and had the program come to completion as originally planned, numerous objectives set out in the grant proposal would have remained unaddressed.

In developing the Tenth Work Plan, the Management Entity (ME) at Oregon State University issued a restricted Request for Proposals (RFP) in February 2001 with an April deadline for proposal submission. The RFP solicited proposals for regional and cross-cutting research and in research areas that were underrepresented in the Eighth and Ninth Work Plans, with the intent to fill gaps in the body of research carried out under the current grant. (For interested readers, the RFP is included in pages 137 to 148 of this document.)

The proposals submitted in response to the RFP were reviewed by experts outside of the program and by CRSP researchers. The Work Plan and Budget Subcommittee of the Technical Committee then evaluated the reviews and made recommendations to the ME. Proposals were selected for funding based on their technical merit, gaps in the current CRSP research portfolio, and geographic regions. Tenth Work Plan funding decisions were announced in July 2001.

A Note on Organization

In the Tenth Work Plan document, investigations are organized by research theme and research area, following the grant framework. This is a departure from the regional vs. cross-cutting research structure used for the Eighth and Ninth Work Plans. Settling on an organizational structure for those work plan collections was straightforward because projects—whether funded to carry out one or five investigations—were easily categorized as either one or the other.

Under the Tenth Work Plan every investigation was required to have a specific host country counterpart and component. To that extent every investigation could reasonably be considered "regional," so the distinction between regional and cross-cutting research projects lost relevance. In addition, a single project might well have received funding to carry out more than one investigation, with each conceivably involving a different host country, so the logic of retaining the grouping of investigations by project disappeared.

Representing the complexity and variety of collaborative relationships among and between the CRSPs participating US and host country researchers and institutions in a regional structure would realistically have necessitated a color-coded, foot-noted, cross-referenced three-dimensional spatial model with detailed instructions and accompanying legend. While such an undertaking would likely have been an intensely satisfying intellectual challenge, using instead the research framework as an organizing principle stood out as the winner in terms of simplicity, clarity, and sheer doability.

Work Plan Reporting

The PD/A CRSP Program Management Office (PMO) at Oregon State University is responsible for, among other things, annual reporting on research progress and accomplishments to USAID. Program investigators in turn have reporting obligations, chief among them being submission of annual reports of technical progress. Progress on the investigations collected here will appear in the PD/A CRSP Twentieth and Twenty-first Annual Technical Reports.

Projects' adherence to work plan schedules and methods and fulfillment of work plan objectives is also tracked to assure continuing accountability for program awards. These types of changes are collected and published in work plan addenda as needed.

Research Areas and Themes Represented in the Tenth Work Plan

Research Theme	Investigation Title	Research Code	Collaborators: US	Collaborators: Host Country	Host Country/ies	Page
	PRODUCTION OPTIMIZATION					
Pond Dynamics	Effects of Pond Age on Bottom Soil Quality	10PDR1	Boyd	Boonyaratpalin	Thailand	9
	Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency	10PDR2	Batterson, Garling, Knud-Hansen	Bart, Grover, Litdamlong, Tuan, Viseth	Bangladesh, Cambodia, Laos, Thailand, Vietnam	13
Feeds and Fertilizers	Nutrition of *Colossoma macropomum* and *Piaractus brachypomus*	10FFR1	C. Kohler, S. Kohler, Lochmann	Alcántara, del Aguila, Tello	Peru	18
	Broodstock Diets and Spawning of *Colossoma macropomum* and/or *Piaractus brachypomus*	10FFR2/2A	Dabrowski, C. Kohler, S. Kohler, Lochmann	Alcántara, del Aguila, Tello	Peru	22
	Polyculture of Grass Carp and Nile Tilapia with Napier Grass as the Sole Nutrient Input in the Subtropical Climate of Nepal	10FFR3	Diana	Lin, Shrestha, Yi	Nepal, Thailand	27
	Development of Economically Feasible Feeds for Semi-Intensive Culture of Tilapia, *Oreochromis niloticus*, Using Locally Available Agricultural By-Products	10FFR4	Lim, Veverica	Gitonga, Liti, Muchiri	Kenya	30
Reproduction Control	Studies on Fate of Methyltestosterone and Its Metabolites in Tilapia and on the Use of Phytochemicals as an Alternative Method to Produce a Monosex Population of Tilapia	10RCR1	Abiado, Dabrowski, Phelps	Contreras, Márquez	Mexico	33
	Selection of a New Nile Tilapia Genetic Line to Provide Broodstock for Southeastern Mexico	10RCR2	Giannico, Schreck	Contreras, Fernández, Márquez	Mexico	38
	IGF as a Growth Rate Indicator in *Oreochromis niloticus*	10RCR3	Brown	Bolivar	Philippines	41
Aquaculture Systems Modeling	Development of a Trophic Box Model to Assess Potential of Ecologically Sound Management for Cove Aquaculture Systems in Tri An Reservoir, Vietnam	10ASMR1	Diana	Hung, Lin, Yi	Thailand, Vietnam	45
New Aquaculture Systems/New Species	Amazon Aquaculture Outreach	10NSR1	C. Kohler, S. Kohler	Alcántara, del Aguila, Tello	Peru	48
	Studies on Reproduction and Larval Rearing of Amazonian Fish	10NSR2/2A	Abiado, Dabrowski, Kohler	Alcántara, Tello	Peru	52
	Survey of Tilapia-Shrimp Polycultures in Vietnam and Thailand	10NSR3A	Fitzsimmons	Yi	Thailand, Vietnam	60
	Stocking Densities for Tilapia-Shrimp Polyculture in Thailand	10NSR3B	Fitzsimmons	Yi	Thailand	61
	Survey of Tilapia-Shrimp Polycultures in Mexico and Honduras	10NSR3C	Fitzsimmons	Contreras	Mexico, Honduras	61
	Stocking Densities for Tilapia-Shrimp Polyculture in Mexico	10NSR3D	Fitzsimmons	Contreras	Mexico	62
	Survey of Tilapia-Shrimp Polycultures in Philippines	10NSR3E	Fitzsimmons	Bolivar	Philippines	63
	Evaluation of Growth and Reproductive Performance of Three Strains of Nile Tilapia *Oreochromis niloticus* Found in Kenya for Use in Aquaculture	10NSR4	Phelps, Veverica	Liti, Muchiri, Omolo	Kenya	65
	ENVIRONMENTAL EFFECTS					
Effluents and Pollution	Reaction of Liming Materials in Pond Bottom Soils	10ER1	Boyd, Wood	Prinsloo, Scholtz, Theron, Queiroz	Brazil, South Africa	68
	Elimination of Methyltestosterone (MT) from Intensive Masculinization Systems: Use of Activated Charcoal in Concrete Tanks	10ER2	Giannico, Schreck	Contreras, Márquez	Mexico	71
	Environmental Impacts of Cage Culture for Catfish in Chau Doc, Vietnam	10ER3	Diana	Lin, Phuong, Yi	Thailand, Vietnam	75

Research Areas and Themes Represented in the Tenth Work Plan (continued)

Research Theme	Investigation Title	Research Code	Collaborators		Host Country/ies	Page
			US	Host Country		
	Environmental Effects (continued)					
Appropriate Technology	Regionalizing Training and Technical Assistance for Nongovernmental Organizations	10ATR1	Molnar, Tollner, Verma	Isaula, Meyer	El Salvador, Honduras, Nicaragua	77
	Institutionalizing Web-based Information System for Tilapia Culture in Latin America	10ATR2	Maldonado, Molnar, Tollner, Verma	Isaula, Meyer	El Salvador, Honduras, Nicaragua, Panama	81
	Diversification of Aquacultural Practices by Incorporation of Native Species and Implementation of Alternative Sex Inversion Techniques	10ATR3	Giannico, Schreck	Contreras, Márquez	Mexico	85
	On-Station and On-Farm Trials of Different Fertilization Regimes Used in Bangladesh	10ATR4	Diana	Lin, Wahab, Yi	Bangladesh, Thailand	90
	Use of Clinoptilolite Zeolites for Ammonia-N Transfer and Retention in Integrated Aquaculture Systems and for Improving Pond Water Quality before Discharge	10ATR5	Batterson, Garling, Knud-Hansen	Bart	Thailand	93
GIS: Planning, Policy, and Global Data Analysis	A Study of Aquaculture Brownfields: Abandoned and Converted Shrimp Ponds in Thailand	10GISR1	Brechin, Diana	Bart	Thailand	98
	Assessing Watershed Ponds for Aquaculture Development in Thai Nguyen, Vietnam	10GISR2	Diana	Lin, Luu, Yi	Thailand, Vietnam	101
	Social and Economic Aspects					
Marketing and Economic Analysis	Optimal (Profit-Maximizing) Target Markets for Small and Medium-Scale Tilapia Farmers in Honduras and Nicaragua	10MEAR1	Engle	Arias, Meyer, Saborio	Honduras, Nicaragua	104
	Development and Evaluation of a Simple Market Feasibility Assessment Methodology	10MEAR2	Engle	Alcántara, Contreras	Mexico (A), Peru (B)	108
	Regional Enterprise Budget and Business Plan Development	10MEAR3	Engle	Muchiri	Kenya	111
	Economic and Risk Analysis of Tilapia Production in Kenya	10MEAR4	Engle	Muchiri	Kenya	114
Adoption/ Diffusion	Institutionalizing Techniques for Building Hillside and Levee Ponds for Water Supply and Aquacultural Development in Latin America	10ADR1	Molnar, Tollner, Verma	Meyer, Pilz	El Salvador, Honduras, Nicaragua	117
Food Security	Income, Food Security, and Poverty Reduction: Case Studies of Functioning Clusters of Successful Small-Scale Aquaculture Producers	10FSR1	Molnar, Tollner, Verma	Meyer, Pilz	Honduras	121
Product Diversification	Characteristics of Fish Buyers Likely to Purchase Farm-Raised Tilapia in Honduras and Nicaragua	10PDVR1	Engle	Arias, Meyer, Saborio	Honduras, Nicaragua	127
	Cost Containment Options for Tilapia Production in Central Luzon, Republic of the Philippines	10PDVR2	Brown	Bolivar	Philippines	130
	Transfer of Production Technology to Nepal for Nile Tilapia, *Oreochromis niloticus*	10PDVR3	Diana	Bart, Rai	Nepal, Thailand	134

Host Countries Participating in Tenth Work Plan Investigations

Host Country	Investigation Title	Research Code	Collaborators: US	Collaborators: Host Country	Page
Mexico	Studies on Fate of Methyltestosterone and Its Metabolites in Tilapia and on the Use of Phytochemicals as an Alternative Method to Produce a Monosex Population of Tilapia	10RCR1	Abiado, Dabrowski, Phelps	Contreras, Márquez	33
	Selection of a New Nile Tilapia Genetic Line to Provide Broodstock for Southeastern Mexico	10RCR2	Giannico, Schreck	Contreras, Fernández, Márquez	38
	Survey of Tilapia-Shrimp Polycultures in Mexico and Honduras	10NSR3C	Fitzsimmons	Contreras	61
	Stocking Densities for Tilapia-Shrimp Polyculture in Mexico	10NSR3D	Fitzsimmons	Contreras	62
	Elimination of Methyltestosterone (MT) from Intensive Masculinization Systems: Use of Activated Charcoal in Concrete Tanks	10ER2	Giannico, Schreck	Contreras, Márquez	71
	Diversification of Aquacultural Practices by Incorporation of Native Species and Implementation of Alternative Sex Inversion Techniques	10ATR3	Giannico, Schreck	Contreras, Márquez	85
	Development and Evaluation of a Simple Market Feasibility Assessment Methodology	10MEAR2A	Engle	Contreras	108
El Salvador	Regionalizing Training and Technical Assistance for Nongovernmental Organizations	10ATR1	Molnar, Tollner, Verma	Meyer (Honduras)	77
	Institutionalizing Web-based Information System for Tilapia Culture in Latin America	10ATR2	Molnar, Tollner, Verma	Meyer (Honduras)	81
	Institutionalizing Techniques for Building Hillside and Levee Ponds for Water Supply and Aquacultural Development in Latin America	10ADR1	Molnar, Tollner, Verma	Meyer (Honduras)	117
Honduras	Survey of Tilapia-Shrimp Polycultures in Mexico and Honduras	10NSR3C	Fitzsimmons	Contreras (Mexico)	61
	Regionalizing Training and Technical Assistance for Nongovernmental Organizations	10ATR1	Molnar, Tollner, Verma	Isaula, Meyer	77
	Institutionalizing Web-based Information System for Tilapia Culture in Latin America	10ATR2	Maldonado, Molnar, Tollner, Verma	Isaula, Meyer	81
	Optimal (Profit-Maximizing) Target Markets for Small and Medium-Scale Tilapia Farmers in Honduras and Nicaragua	10MEAR1	Engle	Arias, Meyer	104
	Institutionalizing Techniques for Building Hillside and Levee Ponds for Water Supply and Aquacultural Development in Latin America	10ADR1	Molnar, Tollner, Verma	Meyer, Pilz	117
	Income, Food Security, and Poverty Reduction: Case Studies of Functioning Clusters of Successful Small-Scale Aquaculture Producers	10FSR1	Molnar, Tollner, Verma	Meyer, Pilz	121
	Characteristics of Fish Buyers Likely to Purchase Farm-Raised Tilapia in Honduras and Nicaragua	10PDVR1	Engle	Arias, Meyer	127
Nicaragua	Regionalizing Training and Technical Assistance for Nongovernmental Organizations	10ATR1	Molnar, Tollner, Verma	Meyer (Honduras)	77
	Institutionalizing Web-based Information System for Tilapia Culture in Latin America	10ATR2	Molnar, Tollner, Verma	Meyer (Honduras)	81
	Optimal (Profit-Maximizing) Target Markets for Small and Medium-Scale Tilapia Farmers in Honduras and Nicaragua	10MEAR1	Engle	Saborio	104
	Institutionalizing Techniques for Building Hillside and Levee Ponds for Water Supply and Aquacultural Development in Latin America	10ADR1	Molnar, Tollner, Verma	Meyer (Honduras)	117
	Characteristics of Fish Buyers Likely to Purchase Farm-Raised Tilapia in Honduras and Nicaragua	10PDVR1	Engle	Saborio	127
Panama	Institutionalizing Web-based Information System for Tilapia Culture in Latin America	10ATR2	Molnar, Tollner, Verma	Meyer (Honduras)	81
Brazil	Reaction of Liming Materials in Pond Bottom Soils	10ER1	Boyd, Wood	Queiroz	68
Peru	Nutrition of *Colossoma macropomum* and *Piaractus brachypomus*	10FFR1	C. Kohler, S. Kohler, Lochmann	Alcántara, del Aguila, Tello	18
	Broodstock Diets and Spawning of *Colossoma macropomum* and/or *Piaractus brachypomus*	10FFR2/2A	Dabrowski, C. Kohler, S. Kohler, Lochmann,	Alcántara, del Aguila, Tello	22
	Amazon Aquaculture Outreach	10NSR1	C. Kohler, S. Kohler	Alcántara, del Aguila, Tello	48
	Studies on Reproduction and Larval Rearing of Amazonian Fish	10NSR2/2A	Abiado, Dabrowski, C. Kohler	Alcántara, Tello	52
	Development and Evaluation of a Simple Market Feasibility Assessment Methodology	10MEAR2B	Engle	Alcántara	108

Host Countries Participating in Tenth Work Plan Investigations (continued)

Host Country	Investigation Title	Research Code	Collaborators		Page
			US	Host Country	
Kenya	Development of Economically Feasible Feeds for Semi-Intensive Culture of Tilapia, *Oreochromis niloticus*, Using Locally Available Agricultural By-Products	10FFR4	Lim, Veverica	Gitonga, Liti, Muchiri	30
	Evaluation of Growth and Reproductive Performance of Three Strains of Nile Tilapia *Oreochromis niloticus* Found in Kenya for Use in Aquaculture	10NSR4	Phelps, Veverica	Liti, Muchiri, Omolo	65
	Regional Enterprise Budget and Business Plan Development	10MEAR3	Engle	Muchiri	111
	Economic and Risk Analysis of Tilapia Production in Kenya	10MEAR4	Engle	Muchiri	114
South Africa	Reaction of Liming Materials in Pond Bottom Soils	10ER1	Boyd, Wood	Prinsloo, Scholtz, Theron	68
Bangladesh	Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency	10PDR2	Batterson, Garling, Knud-Hansen	Grover	13
	On-Station and On-Farm Trials of Different Fertilization Regimes Used in Bangladesh	10ATR4	Diana	Wahab	90
Cambodia	Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency	10PDR2	Batterson, Garling, Knud-Hansen	Viseth	13
Laos	Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency	10PDR2	Batterson, Garling, Knud-Hansen	Litdamlong	13
Nepal	Polyculture of Grass Carp and Nile Tilapia with Napier Grass as the Sole Nutrient Input in the Subtropical Climate of Nepal	10FFR3	Diana	Shrestha	27
	Transfer of Production Technology to Nepal for Nile Tilapia, *Oreochromis niloticus*	10PDVR3	Diana	Rai	134
Philippines	IGF as a Growth Rate Indicator in *Oreochromis niloticus*	10RCR3	Brown	Bolivar	41
	Survey of Tilapia-Shrimp Polycultures in Philippines	10NSR3E	Fitzsimmons	Bolivar	63
	Cost Containment Options for Tilapia Production in Central Luzon, Republic of the Philippines	10PDVR2	Brown	Bolivar	130
Thailand	Effects of Pond Age on Bottom Soil Quality	10PDR1	Boyd	Boonyaratpalin	9
	Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency	10PDR2	Batterson, Garling, Knud-Hansen	Bart	13
	Polyculture of Grass Carp and Nile Tilapia with Napier Grass as the Sole Nutrient Input in the Subtropical Climate of Nepal	10FFR3	Diana	Lin, Yi	27
	Development of a Trophic Box Model to Assess Potential of Ecologically Sound Management for Cove Aquaculture Systems in Tri An Reservoir, Vietnam	10ASMR1	Diana	Lin, Yi	45
	Survey of Tilapia-Shrimp Polycultures in Vietnam and Thailand	10NSR3A	Fitzsimmons	Yi	60
	Stocking Densities for Tilapia-Shrimp Polyculture in Thailand	10NSR3B	Fitzsimmons	Yi	61
	Environmental Impacts of Cage Culture for Catfish in Chau Doc, Vietnam	10ER3	Diana	Lin, Yi	75
	On-Station and On-Farm Trials of Different Fertilization Regimes Used in Bangladesh	10ATR4	Diana	Lin, Yi	90
	Use of Clinoptilolite Zeolites for Ammonia-N Transfer and Retention in Integrated Aquaculture Systems and for Improving Pond Water Quality before Discharge	10ATR5	Batterson, Garling, Knud-Hansen	Bart	93
	A Study of Aquaculture Brownfields: Abandoned and Converted Shrimp Ponds in Thailand	10GISR1	Diana, Brechin	Bart	98
	Assessing Watershed Ponds for Aquaculture Development in Thai Nguyen, Vietnam	10GISR2	Diana	Lin, Yi	101
	Transfer of Production Technology to Nepal for Nile Tilapia, *Oreochromis niloticus*	10PDVR3	Diana	Bart	134
Vietnam	Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency	10PDR2	Batterson, Garling, Knud-Hansen	Tuan	13
	Development of a Trophic Box Model to Assess Potential of Ecologically Sound Management for Cove Aquaculture Systems in Tri An Reservoir, Vietnam	10ASMR1	Diana	Hung	45
	Survey of Tilapia-Shrimp Polycultures in Vietnam and Thailand	10NSR3A	Fitzsimmons	Yi (Thailand)	60
	Environmental Impacts of Cage Culture for Catfish in Chau Doc, Vietnam	10ER3	Diana	Phuong	75
	Assessing Watershed Ponds for Aquaculture Development in Thai Nguyen, Vietnam	10GISR2	Diana	Luu	101

Effects of Pond Age on Bottom Soil Quality

Pond Dynamics Research 1 (10PDR1)/Study/Thailand

Collaborating Institutions
Kasetsart University, Thailand
Mali Boonyaratpalin

Auburn University
Claude E. Boyd

Objectives

This proposal focuses on the PD/A CRSP production optimization objective to increase the overall sustainability of aquacultural production systems through production optimization and the pond dynamics research theme objective to further our understanding of the influence of pond processes on pond productivity. The study will consider pond soil quality and the specific objectives are:

1) Determine relationships between pond age and several key bottom soil quality variables (pH, exchangeable acidity, thickness of S-horizon, bulk density, total sediment depth, lime requirement, organic carbon, and reactivity of organic matter).
2) Evaluate the neutralizing value, particle-size distribution, and calcium and magnesium content of liming materials normally used by fish farmers in Thailand, and use data on soil characteristics and liming materials to improve the lime requirement technique.
3) Compare different methods of pond soil organic matter analyses.
4) Prepare recommendations on pond bottom soil management that consider changes in soil quality as ponds.

Significance

It has been suggested that organic matter increases in bottom soils as ponds age until an equilibrium organic matter concentration is attained (Avnimelech, 1984; Boyd, 1995). Studies have shown that new ponds have lower concentrations of soil organic matter than older ponds, but information on the rate of increase in organic matter over time is lacking (Munsiri et al., 1995, 1996). Organic matter consists of fresh material that decomposes rapidly and older, more stable material that decomposes more slowly (Munsiri et al., 1995). Soil respiration rate measured as carbon dioxide evolution increases with increasing organic matter concentration (Sonnenholzner and Boyd, 2000). However, no studies have considered if the rate of carbon dioxide evolution per unit of organic matter decreases as ponds become older and more stable organic matter accumulates in pond soils. This information is needed to properly assess organic matter concentrations in pond bottom soils.

Many studies of bottom soil organic matter, including studies supported by PD/A CRSP, used an induction furnace carbon analyzer for making the analyses. Few laboratories that do analyses for aquaculture purposes have carbon analyzers, and less expensive procedures for estimating soil organic carbon such as sulfuric acid-potassium dichromate oxidation (Walkley-Black method) or dry ashing are often used (Nelson and Sommers, 1982; Baldock and Nelson, 2000). An evaluation of the different carbon analysis methods is needed so that correlations can be developed to facilitate comparisons of pond soil organic matter data obtained by different methods.

Methods for lime requirement of pond soil samples are available (Boyd, 1995), but these methods have not been adjusted for the depth and bulk density of the S-horizon. Most existing methods for calculating lime requirements of pond soils use values for the average bulk density of agricultural soils to a depth of 15 cm. These procedures will over estimate lime requirement because the bulk density of the S-horizon is much less than that of normal agricultural soil and the S-horizon usually is less than 15 cm deep (Munsiri et al., 1995). Lime requirements also are based on finely-pulverized calcium carbonate with a neutralizing value of 100% (Adams and Evans, 1962; McLean, 1982). Liming materials used by fish farmers may differ greatly from the calcium carbonate standard used for calculating lime requirement.

The acquisition of more precise information on changes in pond bottom soils and data on variation in liming material characteristics are critical for refining pond soil management protocol. These data will reveal if lime requirement computations should be adjusted for pond age and source or type of liming material. Knowledge of the rate of soil organic matter accumulation and any changes in the reactivity of organic matter as ponds age will be valuable in determining how often ponds should be drained and their bottoms dried in order to enhance organic matter decomposition. Findings of the study will be used to develop a pond soil management protocol that considers pond age as a factor.

The study described in this proposal fits well under the Pond Dynamics research theme of the RFP and Continuation Plan. The Pond Dynamics theme specifically mentions the need to characterize pond sediment to obtain information needed in the development of more effective pond management techniques.

Quantified Anticipated Benefits

The overall benefit expected from this project will be the development of some specific soil management practices for use by farmers in Thailand and other countries to improve soil quality in fish ponds.

Specific benefits are expected as follows:

1) More efficient calculations of liming rates and use of liming materials. This should result in an economic savings on liming material and better pond soil pH.
2) More efficient pond dry-out schedule for reducing organic matter accumulation. This will minimize pond "down-time" between crops for some farmers and improve bottom soil condition for other farmers.
3) The data on changes in soil quality with pond age will be useful in educating farmers on pond dynamics. This information will benefit extension personnel and farmers.

Research Design

Pond Facilities: Ponds for use in this work will be located on private fish farms and at fisheries research stations in central Thailand. These ponds will be 2,000 to 5,000 m^2 in area with average depths of about 1 to 1.5 m. Ponds that have only been used for tilapia culture will be sought, and an attempt will be made to select ponds that are similar with respect to stocking densities, fertilization and feeding regimes, and other management inputs. Only ponds that have been in continuous production for a known number of years will be selected. We plan to select at least 25 ponds for use in the study, and if possible, 35 to 40 ponds will be used.

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

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

Soil organic matter will be determined by three techniques: Leco carbon analyzer, dry ashing at 350°C for 8 hr (Jackson, 1958), and the Walkley-Black sulfuric acid-potassium dichromate oxidation (Nelson and Sommers, 1982).

Reactivity of organic matter will be determined in aerobic, laboratory incubation chambers by a carbon dioxide evolution technique described by Sonnenholzner and Boyd (2000). Portions of acidic samples will be treated with agricultural limestone to neutralize exchangeable acidity and reactivity will be determined on both limed and unlimed portions.

Samples of liming material will be analyzed for particle size distribution and neutralizing value by methods presented by Boyd (1995). Samples will be dissolved in acid, and calcium and magnesium determined by EDTA titration using eriochrome black-T and murexide, respectively, as indicators.

Statistical Analysis: The nature of this study does not allow for replication of pond ages as treatments. The data will be analyzed primarily by regression analysis using age as the dependent variable and soil quality variables as independent variables.

Regional Integration

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

Schedule

The tentative schedule follows:

July 2001	Begin project. Trip to Thailand to collect samples of soil and liming materials.
July 2001-May 2002	Analyze samples and resulting data.
June 2002	Trip to Thailand to collect additional samples.
June-December 2002	Finish analyses. Develop better practices based on data.
January 2003	Trip to Thailand to collect samples needed to fill in gaps in data and to refine practices.
February-April 2003	Prepare final report.

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Workshops on Using Principles of Pond Dynamics to Optimize Fertilization Efficiency

Pond Dynamics Research 2 (10PDR2)/Activity/Bangladesh, Cambodia, Laos, Thailand, and Vietnam

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Objectives

The primary objective of the proposed workshops is to transfer existing scientific knowledge, generated through the PD/A CRSP, on how to improve the predictability of pond management and productivity through an understanding of pond dynamics. By teaching host country university, government, and aquaculture extension personnel how to practically apply ecological principles to improve pond fertilization efficiencies, this knowledge can then be transferred to the farmers who will benefit the most. Specifically, the objectives are to teach through demonstrations, presentations, and informal discussions the following topics:

1) Managing factors which control primary and secondary productivities in fertilized ponds;
2) Ecological benefits and limitations of organic and inorganic fertilizers as related to pond dynamics;
3) Pond characteristics that affect fertilization decisions;
4) Methods for determining fertilization requirements; and
5) How to use the algal bioassay test kit for identifying pond- and time-specific fertilization requirements.

Significance

It is well established that the rate of fish production raised on natural foods is directly related to the rate of net algal production (McConnell et al., 1977; Almazan and Boyd, 1978; Liang et al., 1981). This relationship is quite logical, since algal productivity is the energetic foundation for secondary production and detritus formation, and all three are basic and valuable food sources for omnivorous and detritivorous fish (Schroeder et al., 1990). The issue then becomes, how to most efficiently stimulate algal productivity and natural food production in the pond? Over and under fertilizations are wasteful, economically inefficient, and usually lead to lower and more unpredictable fish yields. Efficient fertilization means giving the pond algal community what it needs to grow at that time—nothing more and nothing less.

Factors which generally limit algal productivity in ponds are the availabilities of soluble phosphorus (P), soluble inorganic nitrogen (N), inorganic carbon (C; particularly in rain-fed ponds), and light. If there is an ample supply of P, N, and C, then the algal community will continue to grow until light availability becomes limiting due to self-shading (and/or inorganic turbidity). If one or more of these nutrients are in short supply relative to the needs of the algal community, then algal productivity is said to be limited by that nutrient(s).

These conditions of nutrient and light limitation are time- and pond-specific, particularly in fertilized ponds where N, P, and sometimes C are added in large quantities to stimulate algal growth, and light can be limiting due to sediment resuspension from rain/wind storms or fish activity. What might be appropriate for one pond may be inefficient for the pond next to it. This is particularly true with P, since the age of the pond and the pond's history of prior fertilizations will affect how much of P fertilizer gets adsorbed by the pond's sediments and how much remains available for algal uptake—even during the course of a grow-out period (Knud-Hansen, 1992). In another example, changes from N limitation to C limitation were observed in ponds with low carbonate alkalinities and fertilized with urea (Knud-Hansen et al., 1991). These variable ecological factors, among others (e.g., pond depth, wind exposure, bank stabilization, etc.), make generalized fertilization recipes inefficient, both with respect to stimulating algal growth and to farm economics.

The culture pond can become a more efficient and predictable production system if the farmer, or the person advising the farmer, understands dynamic aspects of pond ecology, including dissolved oxygen dynamics, thermal stratification, primary production and decomposition, and the role of inorganic turbidity. Decisions on where to place the ponds, types of source water, pond depth and surface area, and managing pond sediments are all affected by pond dynamic considerations (Knud-Hansen, 1998). In particular, understanding pond ecology promotes knowledgeable choices of appropriate fertilizers to both optimize fertilization efficiency and minimize unwanted environmental impacts (Knud-Hansen and Pautong, 1993; Knud-Hansen et al., 1993; Shevgoor et al., 1994).

Understanding pond ecology helps the farmer manage ponds, but it does not necessarily identify the nutritional requirements of each pond's algal community. Since fish productivity is directly linked to algal productivity, growing more fish food means growing bigger fish. A simple way to identify what the algal community needs to satisfy its nutrient limitation(s) is with an algal bioassay.

Algal bioassays have been used for decades by limnologists to identify nutrient limitation for lake management, i.e., what not to put in the lake to make it eutrophic (Middlebrooks et al., 1976). The algal bioassay is a simple responsive test where pond or lake water is fertilized (or spiked) with specific algal nutrients, particularly N and P. If the indigenous algal community grows in response to a single nutrient addition, then that nutrient is said to be limiting. If there already is an excess supply of that nutrient in the water, then adding more will not result in an algal growth response. Sometimes both N and P can be limiting when the availabilities of both nutrients are low. In other cases, fertilizing with one nutrient can promote the limitation of another (Knud-Hansen et al., 1991). Pond fertilization fixed-input recipes are designed to avoid this, but variabilities between ponds limit the utility of recipes, and explain why ponds fertilized identically will give an unpredictable range of fish yields.

To account for the effects of these ecological variabilities, the algal bioassay method has been specifically adapted for determining pond fertilization requirements on a pond- and time-specific basis—i.e., to identify what to put in the pond to turn it green (Knud-Hansen, 1998). The algal bioassay method eliminates the inefficiencies of a single recipe because it determines the fertilization needs based on the individual chemical and biological conditions of each pond at that time. For example, if algal growth in a particular pond is limited by N availability, then fertilizing with P is wasteful, regardless of what a fertilization recipe says. The algal bioassay method identifies primary limitation (i.e., the single nutrient which is in least supply to that pond's algal community's requirements), secondary limitation (e.g., when P is only slightly limiting so that the fertilization with P will then make N limiting—in this case P would be fertilized at full rate for that week, while N only at half the rate), co-limitation (e.g., both N and P are limiting, and both are fertilized at full rates), and light limitation (e.g., due to high algal biomass and/or inorganic turbidity, in which case neither N or P or C would be added at that time, since there is insufficient light availability for the algae to utilize the added nutrients).

The algal bioassay method for pond fertilization has been tested under controlled and field conditions, and it has proven superior in terms of nutrient fertilization efficiencies and farm economics to standard fertilization recipes and fertilization requirements determined by computer modeling (Knud-Hansen et al., 1996; Knud-Hansen et al., accepted for publication). The proven benefits of the algal bioassay method are both economic and environmental. Fertilization requirements are fine-tuned on a pond-by-pond basis, so algal productivity is maximized with the minimal amounts of nutrients added. Fish yields are more consistent and predictable, and there is no excess accumulation of N and P in the pond water, so environmental effects upon discharge are minimized. Furthermore, it eliminates the risk of ammonia toxicity since over-fertilization with N is impossible. Lastly, aquaculture extension officers are empowered with the knowledge, confidence, and simple means to improve the economic and environmental sustainability of semi-intensive, pond aquaculture production systems. It does not matter whether the fish culture is monoculture or polyculture, just as long as the culture species utilize natural foods.

Knud-Hansen has developed a portable algal bioassay kit (described below) that requires no water chemistry, no electricity, no computers, and even literacy to use. By teaching people within the university, government extension, and NGO host country aquaculture community the principles of pond dynamics and how to use the algal bioassay test kit, this expertise can then be transferred to the farmers whose lives can be improved through more sustainable fertilization practices.

Quantified Anticipated Benefits

There should be a total of about 90 aquaculture university professors, students, government extension workers, fisheries staff and others in five Southeast Asian countries who will have a working knowledge of how to apply principles of pond dynamics for improving the yields and sustainabilities of semi-intensive aquaculture. The primary economic beneficiaries of the proposed workshops will be aquaculture farmers, who will be able to improve fertilization efficiencies to produce higher yields while minimizing fertilization inputs through the guidance of workshop participants.

Research Design

In order to transfer this knowledge, workshops will be given on pond dynamics and the application of the algal bioassay test kit for determining pond-specific fertilization requirements. Part of the workshop will be informal lectures to discuss principles of pond ecology which have a direct relationship towards efficient pond management.

The workshops will each have about 15-20 participants, and will be given at locations where the PD/A CRSP and/or the Asian Institute of Technology (AIT) aquaculture program have established formal connections:

1) AIT, Bangkok, Thailand, to university aquaculture students and government extension officers;
2) Bangladesh Agricultural University, Dhaka, Bangladesh, to university students and government fisheries staff;
3) Cambodia Department of Fisheries, Phnom Penh, Cambodia, to office staff, university teachers, fisheries station staff, and possibly NGO staff;
4) Regional Development Coordination for Livestock and Fisheries, Savannakhet, Laos, to provincial fisheries staff; and
5) Research Institute for Aquaculture, Bac Ninh, Vietnam, to researchers, university teachers, and extension workers.

The primary source material will the PD/A CRSP publication *Pond Fertilization: Ecological Approach and Practical Applications* (Knud-Hansen, 1998). Since this can be downloaded from the PD/A CRSP website, access to the book is universal. Discussions will not necessarily be at the same technical level as the book, but will still focus on such topics as managing factors which control primary and secondary productivities (e.g., concepts of nutrient limitation), ecological benefits and limitations of organic and inorganic fertilizers as related to pond dynamics, pond characteristics that affect fertilization decisions (e.g., pond location, depth, use of structures such as hapas and cages), and the advantages and disadvantages of current methods for determining fertilization requirements.

Where applicable, demonstrations will be used to illustrate ecological principles. For example a portable fluorometer (which measures both chlorophyll *a* and turbidity) and dissolved oxygen meter (which also measures temperature) will be used to demonstrate diel changes and differences between ponds. These data will then be analyzed during the workshop to illustrate ecological principles of productivity, decomposition, thermal stratification/destratification, and others that can influence fertilization strategies. Each participant will leave with a greater understanding of how a pond works ecologically, how to use that knowledge to improve predictable fish yields with reduced economic/ environmental costs, and how to recognize and prevent pond conditions that will hinder fish production.

The source book also describes in detail the algal bioassay method for determining pond fertilization requirements. Algal bioassay test kits will be provided for each workshop participant. They will gain experience with its application with field demonstrations using ponds fertilized under different loading rates to promote limitations of P, N, C, and light. The test kits will remain in the host country.

The algal bioassay test is very simple to conduct. Pond water is added to nine clear plastic bottles and each spiked with either N, P, C, N+P, N+C, P+C, N+P+C, and de-ionized water (the 9th is the initial). The nutrient spikes can be made from local fertilizers. The eight bottles are incubated in the specially designed kit box either in the pond or on land under indirect sunlight for 2-3 days to allow pond algae to respond to specific nutrient enrichments. After incubation, a measured sample from each flask is filtered, and filter colors are compared visually. If colors are not distinguishable, then there is likely light limitation. There are only 21 possible filter color combinations. A chart is provided to determine from the specific filter-color combination for that test how that pond should be fertilized for that week. The portable test kit provides the incubation container, filtering apparatus (hand filter using a 50 mL plastic syringe and a Millipore Swinnex filter holder—filters can also be punched out of paper coffee filters), charts, and all other necessary materials that are either made of “unbreakable” plastic or are locally available. Continued use of the test kit will not result in any reliance on foreign materials/ supplies.

Regional Integration

The Regional Plan for Southeast Asia strongly encourages strengthening current linkages with AIT and the PD/A CRSP to neighboring countries. These workshops to be conducted in Bangladesh, Cambodia, Laos, and Vietnam will facilitate that goal. Knud-Hansen, who will be giving the workshops, has had associations with the PD/A CRSP since 1985, and with AIT since 1988. He will continue to work and collaborate with PD/A CRSP and AIT researchers through this proposal as well. These workshops also provide a forum for extending scientific knowledge gained from years of PD/A CRSP research beyond the borders of PD/A CRSP host countries. These workshops will help solidify the informational and research networks necessary to best achieve the goals of the PD/A CRSP. Information dissemination and regional integration are the two main benefits of these workshops.

Schedule

The five proposed 4-day workshops will take place between January and May 2002, which has already been found acceptable to the participating institutions. The exact timing of each workshop will be coordinated to coincide with Knud-Hansen’s two trips to AIT to conduct the zeolite technology experiments described in the work plan for 10ATR5. Attempts will be made to schedule two workshops during the first trip and the other three workshops during the second trip. All travel to Bangladesh, Cambodia, Laos, and Vietnam will originate from Bangkok, Thailand. Final report will be submitted no later than 31 July 2002.

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Nutrition of *Colossoma macropomum* and *Piaractus brachypomus*

Feeds and Fertilizers Research 1 (10FFR1)/Experiment, Study, Activity/Peru

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Objectives

1) Compare aquaculture performance of *Colossoma* and/or *Piaractus* fed formulated diets at varying protein levels.
2) Assess the feasibility of utilizing native Amazonian plant products for small-scale sustainable aquaculture production of *Colossoma* and *Piaractus.*
3) Assess and compare the plant seed dispersal potential of *Colossoma and Piaractus.*

Significance

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

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

Quantified Anticipated Benefits

The development of sustainable aquaculture of *Colossoma* and/or *Piaractus* will benefit many sectors throughout the Peruvian Amazon. Rural farmers will benefit by the addition of an alternative form of agriculture. Aquaculture production will require considerably less land than that needed for cattle ranching. Moreover, ponds can be used year-after-year whereas rain forest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons. Such lands, once abandoned, usually can no longer support normal jungle growth. Both rural and urban poor will benefit by the addition of a steady supply of high quality protein in the marketplace. Aquaculture of *Colossoma* and/or *Piaractus* should relieve some of the fishing pressure on these overharvested, native species. The project will provide economic benefits to large-scale farmers by developing efficacious prepared diets and to small-scale farmers by developing a feeding regime using locally available plant products. *Colossoma* and/or *Piaractus* have been theorized to play a crucial ecological role in disseminating seeds from the flooded forest. This project will provide experimental evidence related to this theory. If the theory proves to be true, the aquaculture of *Colossoma* and/or *Piaractus* would be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Peruvian Amazon.

Research Design and Activity Plan

Experiment A: Compare Aquaculture Performance of *Colossoma* and/or *Piaractus* Fed Formulated Diets at Varying Protein Levels (Objective 1)

Performance of *Colossoma* and/or *Piaractus* fed a formulated feed at three protein levels will be evaluated. Diets will be formulated with protein levels at 22, 27 and 32% using the same ingredients as the diets used for the Ninth Work Plan (fishmeal, soybean, wheat, rice, cornmeal, vitamin C, vitamin/mineral premix, and fish oil). Nine ponds at either the IIAP or UNAP aquaculture stations (3 treatments with 3 replicates) approximately 0.125 ha in size will be stocked at a rate of 8,000 25-g fingerlings per hectare. In the Ninth Work Plan, in the *Piaractus* stocking density experiment, no statistically significant differences in aquaculture performance occurred within the three treatments (4,000; 6,000; and 8,000 fingerlings/ha). Prior to stocking, ponds will be filled with runoff water and limed (0.1 kg/m^2). Ponds will be fertilized at stocking with chicken manure (0.1 kg/m^2) and green grass (0.15 kg/m^2). Fish will be fed twice daily at 10 am and 2 pm. Growth will be monitored over a six-month period. Water quality parameters (pH, D.O., nitrite, nitrate, ammonia, CO_2, and chlorides) will be monitored weekly and temperature and transparency (Secchi disk; cloud-free days at noon) measured daily.

Fish will be sampled bi-weekly and weighed to adjust food rations. At harvest, survival (%), specific growth rate, standing crop at harvest, condition (K), and feed conversion efficiency will be calculated. Data values will be analyzed by one-way analysis of variance (ANOVA). Appropriate transformations will be made where necessary. If significant differences among treatment means are found, the appropriate post-hoc test will be employed to determine where the differences lie. The accepted level of significance will be 0.05.

Study B: Assess the Feasibility of Utilizing Native Amazonian Plant Products for Small-Scale Sustainable Aquaculture Production of *Colossoma* and *Piaractus* (Objective 2)

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

Activity C: Assess and Compare the Plant Seed Dispersal Potential of *Colossoma* and *Piaractus* (Objective 3)

The theory by Goulding (1980) about seed dispersal by *Colossoma* and *Piaractus* will be tested to demonstrate the potential importance of *Colossoma* and *Piaractus* in riverine ecosystems. Diverse size fruits with seeds from *Psidium guajaba*, *Hevea brasiliensis*, *Bactris gassipae*, *Piranhea trifoliata*, *Pseudobombax munguba*, *and Myrciaria cauliflora* will be fed to *Colossoma* and *Piaractus*. For each type of fruit selected, the number of seeds per kilogram of fruit will be estimated. The experiment will take place in six tile-covered raceways (1.2 × 0.8 × 0.8 m). Three adult *Colossoma* and three adult *Piaractus* (each > 7.5 kg in size) will be randomly stocked in each raceway. Prior to starting the experiment, the *Colossoma* and *Piaractus* will be starved for two days to allow for cleansing of their digestive tracts. Each fish will be fed a given fruit singularly to satiation. Feces will be collected each day and intact seeds separated. The number of seeds passing through the fish will be compared with the number of seeds originally estimated to have been fed. Seeds passing through the fish intestines and controls (seeds collected directly from plants) will subsequently be planted under the same environmental conditions (light intensity, temperature, photoperiod, etc.) in oven-pasteurized humus. The viability and germination rate of any intact seeds passing through fish intestines will be compared with the seeds of the unconsumed fruit. Data values will be analyzed by one-way analysis of variance. This experimental design will be conducted with all six fruits with both species of fish.

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

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

N.D. = No data available

Regional 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.

Schedule

Activities for Objective 1 will take place March to November 2002. Activities for Objective 2 will take place July 2001 to March 2003. Activities for Objective 3 will take place July 2001 to December 2002. A final report will be submitted on or before 30 April 2003.

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Broodstock Diets and Spawning of *Colossoma macropomum* and/or *Piaractus brachypomus*

Feeds and Fertilizers Research 2 and 2A (10FFR2 and 2A)/Experiment/Peru

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Objectives

1) Compare the effects of isocaloric high (40%) and low (30%) protein diets on reproductive performance of *Colossoma* and/or *Piaractus* broodstock as indicated by biochemical composition of plasma, ovules, and semen.
2) Compare spawning performance and annual cycles of blood plasma steroid concentrations for *Colossoma* and/or *Piaractus* broodstock fed isocaloric high (40%) and low (30%) protein (anchovy fish meal) diets.

Significance

A need exists to 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. Male and female *C. macropomum* generally reach sexual maturity in 3 and 4 years, respectively, when they have attained 3 to 6 kg in weight. *Piaractus* become sexually mature about a year sooner, and at a smaller size (2 to 4 kg). Fish held in captivity must be induced to spawn using hormones (Gonzales, 1987). After hormonal treatment gametes are stripped and mixed using the dry method (Alcantara and Guerra, 1992). Both MacDonald-type and Woynarovich-type incubators are employed, with the latter being most common. Hatching occurs 10 to 20 h post-fertilization at 26 to 29°C.

Research on spawning and grow-out of gamitana *Colossoma macropomum* and paco *Piaractus brachypomus*, two important foodfish in Peru, were initiated in the Ninth Work Plan. One of our specific objectives was to determine the changes of plasma sex steroid hormones occurring prior to ovulation or spermiation in *P. brachypomus*. For this purpose, we induced ovulation and spermiation using LHRHa. The response patterns of plasma sex steroids to the hormonal treatments were similar in both genders. The concentrations of testosterone and 17,20β-dihydroxy-4-pregnen-3-one significantly increased (Dabrowski et al., 2001). However, we observed that the levels reached under normoxic conditions were higher than the ones recorded under hypoxia. Hypoxia resulted also in significantly lower survival of embryos (17.3 ± 28%) in comparison to normoxic conditions (68.5 ± 25%). Therefore, the complete spawning failure observed at the Instituto de Investigaciones de la Amazonia Peruana (IIAP) station in Iquitos during the previous years may be related in part to low concentration of oxygen in the water in which the broodstock are stocked prior to spawning as well as the hypoxia (below 4 mg O_2/L) during the egg incubation.

During the Ninth Work Plan, we successfully induced ovulation and spermiation of *P. brachypomus* using LHRHa. Both genders were injected with two doses of LHRHa. The concentration of preparation was 0.0042 mg of equivalents of active hormone per ml. Males and females were injected with 1 ml/kg and 2.6 ml/kg, respectively. The priming dose (50% and 10% in males and females, respectively) was administrated in the morning, whereas the resolving dose (50% and 90% in males and females, respectively) was injected at 2200 h. Oviposition was observed within 8 to 16 hours following the resolving dose of the hormone and survival at 13 hours of incubation amounted to 68.5 ± 25. In the Tenth Work Plan, we will determine the effect of improved broodstock nutrition on maturation and spawning performance of *C. macropomum* and/or *P. brachypomus*.

Nutrition is known to affect reproductive success in fishes (De Silva and Anderson, 1995), but the effects are not well documented in colossomids. Past spawning failures of captive colossomids in Iquitos in the past could be due partly to poor nutrition. The diet used currently to maintain broodstock in Iquitos contains about 32% protein, which is intermediate between the requirements for larval and adult colossomids (Araujo-Lima and Goulding, 1997). However, the diet appears to contain a suboptimal amount of total energy compared to diets used in most feeding experiments with colossomids. Insufficient levels of non-protein dietary energy can cause excessive protein catabolism to meet energy requirements, resulting in loss of essential amino acids for other critical functions such as gamete formation. After spawning, the developing larvae are dependent mostly on proteins of maternal origin to continue normal growth and development until exogenous feeding begins. Therefore, the dietary protein and energy:protein ratios of the broodstock diets could be a major determinant of spawning success and larval quality in colossomids. In this study, reproductive performance of colossomids fed two diets similar in total energy but differing in crude protein content (30 or 40%) will be compared in a field trial to that of broodstock fed the diet currently in use in Iquitos for two spawning seasons. Reproductive performance will be assessed using biochemical data from plasma, ovules and semen of broodstock fed the different diets.

Quantified Anticipated Benefits

This project will result in improved nutrition for broodstock of two important foodfishes in the Peruvian Amazon, *Colossoma macropomum* and *Piaractus brachypomus*. Spawning performance is expected to significantly improve for both species as an outcome of the research. The further development of sustainable aquaculture of these two species will benefit many sectors throughout the Peruvian Amazon. Rural farmers will benefit by the addition of an alternative to other forms 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, and 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/or *Piaractus* should relieve some of the fishing pressure on these overharvested, native species. These species have been theorized to play a crucial ecological role in disseminating seeds from the flooded forest. Accordingly, the aquaculture of *Colossoma* and/or *Piaractus* could be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Peruvian Amazon.

Research Design

Objective 1: Compare the Effects of Isocaloric High (40%) and Low (30%) Protein Diets on Reproductive Performance of *Colossoma* and/or *Piaractus* broodstock as Indicated by Biochemical Composition of Plasma, Ovules, and Semen

Two practical diets will be formulated for colossomid broodstock with ingredients commonly used in fish diets in Iquitos, except that palm oil (4%) will be added to each diet to increase the available energy, carotenoid and alpha-tocopherol levels. Palm oil has been used successfully in *Colossoma* diets previously (Viegas and Guzman 1998). The diets will also be similar in total calorie content, but will differ in total protein content (30 or 40%). The composition of the diets is shown in Table 1. The proportions of feedstuffs in the diets were manipulated to achieve the desired total protein and energy levels. The diets will be prepared in Iquitos, Peru, and their proximate composition will be verified analytically at the University of Arkansas at Pine Bluff. The amino acid and fatty acid profiles of the diet also will be analyzed.

Table 1. Composition (%) of diets for colossomid broodstock .[A]

Ingredient	30% Protein[B]	40% Protein[B]
Fish meal	20.00	28.00
Soybean meal	30.00	50.00
Wheat husks	28.00	18.00
Corn flour	16.00	0.00
Vitamin/Mineral premix	1.95	1.95
Vitamin C	0.05	0.05
Palm Oil	4.00	4.00

[A] The calculated energy to protein ratios of the diets with 30 and 40% protein are 10.7 and 8.7 kcal/gram dietary protein, respectively, based on digestible energy values for channel catfish or physiological fuel values (NRC, 1993).
[B] Total dietary protein levels calculated using analyzed protein content of the individual ingredients.

These two new diets will be compared to the broodstock diet currently in use by comparing the reproductive performance of fish fed the different diets in a field trial (described under objective 2). Reproductive performance for objective 1 will be assessed by measuring and comparing the amino acid and fatty acid composition of the ovules and semen from fish fed the different diets. Lipid class composition of ovules, semen and plasma also will be determined by use of an iatroscan at the University of Arkansas at Pine Bluff using methods described previously (Lochmann et al., 1995). Samples will be collected at the same time as those collected for objective 2. Biochemical data will be compared to literature values and to data gathered for objective 2 of this study to determine correlations among indicators of reproductive success in colossomids.

Objective 2: Compare Spawning Performance and Annual Cycles of Blood Plasma Steroid Concentrations for *Colossoma* and/or *Piaractus* Broodstock Fed Isocaloric High (40%) and Low (30%) Protein Diets

Colossoma macropomum and/or *Piaractus brachypomus* broodstock (whichever species is most available) will be raised in three ponds at the IIAP Quistococha Aquaculture Station. Broodstock in one pond will be fed the standard diet that has been used at the station for several years. Fish in another pond will receive the formulated 30% crude protein broodstock diet, while the fish in the third pond will receive the 40% crude protein diet. Water temperature and dissolved oxygen will be recorded daily for each pond. At the beginning of the gonad recrudescence, twenty-five fish will be captured, individually measured, weighed and tagged (PIT-tags, Biosonic, Seattle, WA). Fish will then be released into their respective ponds. Blood will be collected every 3-6 months from the caudal vessel of unanesthetized fish using a heparinized syringe. Blood will centrifuged at 1,500 g for 15 min and the plasma stored at –20°C until assayed. At the time of maturation (normal spawning time in Peru is November to early January), spermiating males and robust females will be selected. Ovarian maturity will be assessed with a microscope using oocytes collected from the ovary with a catheter. Pairs of fish will be moved into indoor 0.75-m^3 concrete tanks. In each tank, the male will be separated from the female by a net. Intensive aeration of the water will be provided upon fish arrival. Both genders will be injected with two doses of LHRHa to induce ovulation and spermiation as described in section D (Significance). Blood will be collected prior to the priming injection and after ovulation or spermiation as described previously (Dabrowski et al., 2001). Semen and ovules will be collected by stripping after anesthesia (Cardoso et al., 1995). Sperm concentrations will be estimated microscopically using a Double Neubauer Counting Chamber and then spectrophotometrically to develop an appropriate equation (Ciereszko and Dabrowski, 1993). The sperm motility will be evaluated by the same observer and recorded as percent motile sperm. Time of ovulation, fecundity and egg size will be recorded in all females. To test the quality of the eggs, ovules from individual females will be fertilized with pooled semen from three to four males and incubated in separate vertical (conical) incubators of 60 L capacity. Egg production/ fish kg will be measured. The rate of survival will be assessed at hatching (19 h at 25°C). Hatching success of the embryos will be calculated and expressed as a percentage of the initial number. The plasma concentrations of steroids (testosterone, estradiol-17β, 11-ketotestosterone and 17,20β-dihydroxy-4-pregnen-3-one) will be measured by radioimmunoassay similar to those used previously (Ottobre et al., 1989) following ethyl-ether extraction. Validation of those steroid assays has already been accomplished in Ohio State University for rainbow trout (Dabrowski et al., 1995), paco (Dabrowski et al., 1997), yellow perch (Ciereszko et al., 1997), lake whitefish (Rinchard et al., 2001) and muskellunge (Dabrowski et al., 2000). Assays for those four steroids will be validated in a similarly rigorous fashion.

Regional Integration

Research efforts being proposed are logical initial steps toward developing sustainable aquaculture in the region. The research will benefit the entire region by providing pertinent information on broodstock nutrition and reproduction. Alcantara will be involved in dissemination of both fish produced during this project and information related to production technologies. This experiment contributes to the regional objective of "further refinements in culture technology for *Colossoma* and/or *Piaractus*" stated in the Regional Plan for South America.

Schedule

All activities will take place from 1 July 2001 through 30 April 2003. The broodstock diets will be formulated and made between 1 July and December 2001. Broodstock blood plasma steroid samples will be collected starting 1 July 2001 on a quarterly basis starting 1 July 2001. Broodfish will be spawned in November and December in years 2001 and 2002 (only 2002 spawn will be with fish fed new experimental diets). A final report will be submitted on or before 30 April 2003.

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Polyculture of Grass Carp and Nile Tilapia with Napier Grass as the Sole Nutrient Input in the Subtropical Climate of Nepal

Feeds and Fertilizers Research 3 (10FFR3)/Experiment/Nepal and Thailand

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Objectives

1) Evaluate the growth of grass carp and tilapia fed with napier grass in polyculture.
2) Evaluate the nutrient and water quality regimes of pond water.
3) Determine the composition of foods consumed by Nile tilapia.
4) Determine the optimal ratio of grass carp to Nile tilapia in polyculture.

Significance

Grass carp *Ctenopharyngodon idella*, a herbivorous species, is a commonly cultured species in many parts of the world, especially in East Asia. In China, polyculture of grass carp with other species of different feeding habits is traditionally practiced, whereas grass carp consume low value vegetative waste and increase natural food production in the pond by nutrient recycling and fecal production (Yang et al., 1990; Li and Mathias, 1994). The effectiveness is depicted in a Chinese saying "one grass carp raises three silver carp." It was reported that a 5:1 stocking ratio by weight is most suitable for grass carp and filter-feeding species in a polyculture system consisting of silver carp *Hypophthalmichthys molitrix*, bighead carp *Aristichthys nobilis*, and common carp *Cyprinus carpio* (Yang et al., 1990). However, as grass carp are known to feed on a wide variety of plants, the quantity and quality of natural food production derived from recycling of grass carp wastes depend largely on the type and input of forage provided.

In Nepal, pond fish culture is mostly conducted in the southern subtropical region, where pond water temperature falls between 15-20 C during winter period from mid-November to mid-February (Shrestha 1999). Polyculture of herbivorous carps is a common practice in Nepal. The major constraints for small-scale, resource-poor farmers are fish feeds and chemical fertilizers, which are expensive and unavailable, while livestock manure is traditionally used for land crops (Shrestha and Yadav, 1998; Shrestha 1999). Exploration of easily available or easily grown plant material that is not used in human food production is a prime need to solve the problems of these fish farmers. Napier grass *Pennisetum purpureum* is a high yielding and perennial tropical grass (Humprey, 1978; Edwards, 1982) that is accepted by grass carp and can produce a reasonable yield (Venkatesh and Shetty, 1978; Shrestha and Yadav, 1998; Shrestha, 1999). As in Chinese polyculture systems, a major portion of plant biomass consumed by grass carp returns to the pond as organic manure which stimulates plankton production for other planktivorous fish in the same ponds (Woynarovich, 1975).

Recently, Nile tilapia *Oreochromis niloticus* was introduced to Nepal and has been cultured in experimental scale (Shrestha and Bhujel, 1999). Nile tilapia is an excellent candidate to be polycultured with grass carp to utilize the natural foods derived from plants fed to grass carp. Polyculture of grass carp and Nile tilapia may have an additional advantage due to the fact that large grass carp can prey to some extent on tilapia fry spawned in the pond (Spataru and Hepher, 1977). To fully utilize available resources, this system should be tested and the ratio of grass carp to Nile tilapia should be evaluated in polyculture ponds.

Anticipated Benefits

The results of this study will investigate a new polyculture system in Nepal as an alternative strategy to culture of grass carp by small-scale farmers. It will not only apply to Nepalese systems, but may also benefit fish culturists in many other countries where grass carp and Nile tilapia are commonly cultured.

Research Design

Location: Institute of Agriculture and Animal Science (IAAS), Nepal

Methods: Pond research

Pond Facility: 15 cement tanks (5x4.8x1.5 m) will be used.

Culture Period: 180 days

Stocking Density: Grass carp (100 g size) at 0.50 fish/m^2, Nile tilapia (10 g size) at 0, 0.25, 0.50, 1.0, and 2.0 fish/m^2.

Test Species: Grass carp *Ctenopharyngodon idella* and Nile tilapia *Oreochromis niloticus*

Nutrient Inputs: Chopped napier grass *Pennisetum purpureum* ad libitum

Water Management: Maintain at 1.5 m depth

Sampling Schedule

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

Fish growth: monthly and total harvest

Gut contents: ten grass carp and ten Nile tilapia from each pond will be sampled randomly from fish removed at harvest and used for gut content analysis.

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

Statistical Design, Null Hypothesis, Statistical Analysis: Experimental design: The experiment is a randomized complete block design. There will be a constant stocking density of grass carp, and a control and four stocking ratios of Nile tilapia. Each treatment will be triplicated. The treatments will be as follows:

A) grass carp only at 0.5 fish/m^2 (50 g/m^2) (control)
B) grass carp plus Nile tilapia stocked at 0.25 fish/m^2 (2.5 g/m^2)
C) grass carp plus Nile tilapia stocked at 0.5 fish/m^2 (5 g/m^2)
D) grass carp plus Nile tilapia stocked at 1 fish/m^2 (10 g/m^2)
E) grass carp plus Nile tilapia stocked at 2 fish/m^2 (20 g/m^2)

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

The null hypothesis is that stocking ratio of grass carp to Nile tilapia has no effect on the growth of grass carp and Nile tilapia, nutrient utilization efficiency, or water quality.

Regional Integration

Nile tilapia is commonly cultured throughout Asia, while grass carp is regarded as high valued fish in China, Thailand, Vietnam and Laos. The successful polyculture of grass carp and Nile tilapia using napier grass will provide an alternative strategy for small-scale resource-poor farmers. It will be used by the AIT outreach program as a possible extension method to regional farmers.

Schedule
April to October 2002
Report submission: January 2003

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Development of Economically Feasible Feeds for Semi-Intensive Culture of Tilapia, *Oreochromis niloticus*, Using Locally Available Agricultural By-Products

Feeds and Fertilizers Research 4 (10FFR4)/Experiment/Kenya

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Objectives

1) Formulate cheap practical diets using locally available agricultural by-products for semi-intensive culture of *Oreochromis niloticus*.
2) Evaluate, under a semi-intensive culture system, the growth performance of *O. niloticus* fed these formulations as compared with that of fish fed maize and starter/broiler diet.
3) Determine the economic feasibility of using these diets as supplement to natural food in a semi-intensive *O. niloticus* production system.
4) Train young undergraduate and post-graduate fellows research skills in aquacultures, feed formulation and processing, and feeding practices.

Significance

Feed represents the largest expenditure item in semi-intensive and intensive culture systems and protein is the most expensive macro-nutrient in fish feeds. In semi-intensive tilapia farming where ponds are heavily fertilized, natural food organisms contribute significant amount of nutrient necessary for fish growth. Compounded feeds formulated for other species have been used as supplement to natural food to increase fish yield. However, due to the availability and high costs of compounded diets, a number of studies have been conducted to evaluate alternative cheap feed supplements for Nile tilapia farming. Such supplements that have been evaluated include rice bran, wheat bran, cassava meal and corn meal (Perschbacher and Lochmann, 1995; Cao et al., 1997; Veverica et al., 1999).

The problem with single ingredients like brans is that they are deficient in both macro and micro-nutrients while the high content of crude fiber in some brans may greatly reduce the digestibility thus, leading to low fish yields (Liti et al., 2001). However, preliminary studies conducted at Sagana fish farm have shown that the performance of different brans in promoting fish growth differed considerably. Maize bran, for example, gave the highest fish yield followed by wheat bran, while rice bran yielded the least. An alternative management practice to increase the fish yield is to use formulated diets.

In an attempt to seek ways of increasing fish yields, a recent study was conducted at Sagana fish farm to compare locally available rice bran with two compounded feeds, a domestic animal feed (pig finisher pellet) and a laboratory formulated diet (Liti et al., 2001). The results demonstrated that the two compounded diets had similar nutritional value and promoted better fish growth than rice bran.

Cao et al. (1997) compared the growth performance of *O. niloticus* fed 20% crude protein diets formulated from chicken feed and fish meal with single ingredients (corn meal and rice bran). The fish in this study performed better with formulated diets as compared to single ingredients. Also an economic comparison performed in this study favored the utilization of formulated diets.

Since the cost of feed represent the major expenses in semi-intensive tilapia production, reducing the cost of feed is necessary to increase profit. Compounded diets for pigs and starter/broiler diets, although locally available and have been shown to provided better growth of tilapia than brans, are very costly. Thus, lower-cost compounded diet formulated from locally available ingredients for semi-intensive production of *O. niloticus* in earthen ponds must be developed.

Quantified Anticipated Benefits

Twenty-five farmers will be provided with information on how to formulate economically effective practical diets at their farms. They will be reached through a workshop to be conducted later after the experiment. The results of the study will help improve profitability in the production of *O. niloticus*, as determined by enterprise budgets, and will reduce reliance on feeds that are available only through special orders and at exaggerated prices. This knowledge will also empower farmers to become strong decision makers in the fish feed industry. Fish yield is expected to increase and the cost of feed is expected to be markedly reduced. For example, a 70-kg bag of commercial pig finisher pellet costs KSh 1,200 (US$15.60) while the costs of broiler starter and maize bran are KSh 1,400 (US$18.20) and 300 (US$3.90) respectively per 70 kg. A 70-kg bag of feed formulated from Nile perch filleting waste meal, cottonseed cake, sunflower oil meal residue, and maize bran to contain crude protein similar to that of starter/broiler feed would cost only KSh 850 (US$11.00) prior to pelleting. This is considerable cheaper than pig finisher pellets and starter/broiler feed.

Research Design

The project will be conducted at Sagana Fish Farm, located at Sagana town which is 90 km north of Nairobi and located at an altitude of 1,230 m, latitude 0°39'S and longitude 37°12'E. Sixteen 800-m^2 static water earthen ponds will be stocked with sex reversed male *O. niloticus* (10.0 g) at a rate of 2 fish/m^2 (20,000 fish/ha). To control snails and to provide a second marketable species, 1,000/ha of fingerling *Clarias* catfish (5.0 g) will also be stocked. This stocking rate was previously shown to effectively control snail populations in station ponds. Prior to stocking and at every two weeks, ponds will be fertilized with inorganic fertilizers at rates previously determined at the site (20 kg and 5 kg of N and P/ha, respectively).

Two experimental diets will be formulated to contain approximately 20% crude protein and 6% fat. Diet 1 will consist of 8% Nile perch filleting waste, 20% cottonseed cake, 15% sunflower seed oil residue and 58% maize bran. Diet 2 will have the same composition as diet 1 except that 0.5% of fish vitamin premix will be added at the expense of maize bran. Available commercial starter/broiler diets will also be evaluated. Maize bran will be used as the control diet. All diets (except maize bran) will be processed into sinking pellets using belt driven mixer and extruder, broken down into suitable sizes and air dried to a moisture content of about 10%. Each diet will be fed to tilapia in four replicate ponds two times daily, once in the morning and once in the afternoon, at a daily rate of 2% of body weight. At least 10% of the total number of tilapia stocked in each pond will be sampled twice a month to determine the average weight and adjust the feeding rate. The ponds will be drained and harvested after 8 months of culture or until fish reach a marketable size of 300 g, whichever comes first.

Fifty fish will be randomly selected at the beginning of the experiment and stored frozen at –8°C for determination of whole body proximate composition. At the conclusion of the feeding study, 10 fish from each pond will be randomly collected and stored frozen for subsequent measurement of body composition. Experimental diets will also be analyzed for chemical composition. Proximate analysis of fish and feeds swill be done following the standard method by AOAC (1990).

Weather data will be gathered from a meteorological station located at the farm. Morning and afternoon water temperature and dissolved oxygen at about 25 cm deep will be measured weekly at the start of the trial and more frequently as the experiment progressed. Total alkalinity, chlorophyll *a*, Secchi disk visibility, total ammonia-nitrogen, soluble reactive phosphorus, nitrate-nitrogen, nitrite-nitrogen, and total hardness will be measured once every two weeks. Total nitrogen and total phosphorus and will be analyzed monthly. All sampling and analyses of samples will be carried out according to the standard CRSP protocols.

The economic assessment of the diets will be determined by enterprise budget analysis, incorporating returns and variable costs (such as fingerlings, feedstuffs, transportation, labor).

Null hypothesis: There are no significant differences in the performance of all diets in terms of growth, total production, feed and protein efficiency, apparent protein utilization, whole body composition and production cost.

Statistical design and analysis: The experimental units (800-m^2 ponds) will be assigned randomly using a completely randomized design with four replications/treatment. All data will be analyzed by a one-way analysis of variance (SAS Institute, 1993). Duncan's multiple range test will be used to determine differences between treatment means. Differences will be considered significant at the 0.05 probability level.

Regional Integration

This proposed research form part of regional activities in Malawi, where the main research activity is pond management and nutrient inputs as means to increase fish production.

Schedule

Preparation of ponds (draining and liming and fertilization), analysis of feed ingredients, and diet formulation and processing, July 2001. (Throughout the course of the study, due to the small capacity of the mixer and extruder, feed processing will be done as needed.)
Stocking of ponds with male sex-reversed *O. niloticus* and *Clarias* catfish, August 2001.
Harvesting of ponds, April 2002.
Chemical analysis of experimental feeds and fish, May to June 2002
Data analysis and final report submission, by 30 April 2003.

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Studies on Fate of Methyltestosterone and Its Metabolites in Tilapia and on the Use of Phytochemicals as an Alternative Method to Produce a Monosex Population of Tilapia

Reproduction Control Research 1 (10RCR1)/Experiment/Mexico

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Objectives

1) Determine concentration of methyltestosterone derivatives in tilapia and water using radioimmunoassay and HPLC methods.
2) Evaluate potential action of phytochemicals on sex differentiation of tilapia.

Significance

In tilapia aquaculture, all-male populations are desirable because males demonstrate superior growth characteristics compared to females. Moreover, culture of monosex populations prevents reproduction and results in uniformity of fish size. The synthetic steroid, 17α-methyltestosterone (MT) is a derivative of a male specific hormone commonly used to masculinize tilapia juveniles (Green et al., 1997; Abucay and Mair, 1997; Gale et al., 1999). The effect of MT is dependent on various factors such as dose, timing and duration of treatment, and mode of administration (Mirza and Shelton, 1988).

The uptake and depletion of MT have been reported in several species of teleost fish, including cichlids (Fagerlund and Dye, 1979; Goudie et al., 1986; Curtis et al., 1991; Cravedi et al., 1993, Rinchard et al., 1999). As MT administrated orally is readily metabolized, research on the fate of MT and on its metabolites need to be addressed for human and environmental safety issues. In the Tenth Work Plan, we propose to determine the levels of MT and its metabolites in tilapia and water using radioimmunoassay (Rinchard et al., 1999) and high-performance liquid chromatography/mass spectrometry (Williams et al., 2000) methods. The MT antiserum that we used for RIA cross-reacted with testosterone (12.6%), dihydrotestosterone (2.8%) and also with some metabolites of MT identified by Cravedi et al. (1993) such as 17α-methyl-5βandrostan-17β-ol-3-one (48%) and 17α-methyl-5αandrostan-3-17β-diol (12.6%). However, cross-reactivities with circulating androgens are thought to have little impact on our estimates of MT concentrations because the concentration of MT in the plasma of fish not supplemented with MT was negligible (Rinchard et al., 1999).

Another problem associated with the use of MT is that, at high doses or prolonged treatment, 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 might be due more to aromatization than to inhibition of in vivo synthesis of androgens. However, these authors stressed that in some species aromatization and inhibition of *in vivo* synthesis of androgens could be the causative factor. Therefore alternative methods to produce monosex populations should be considered.

One approach may involve the use of plant extracts. Gastric intubation of aqueous extracts of *Hibiscus macranthus* and *Basella alba* in rat had anabolizing and virilizing effects (Moundipa et al., 1999). Isoflavonoids such as genistein act as estrogen agonists via estrogen receptors in cultured cells and also manifest estrogen-like effects in the female reproductive system (Miksicek, 1995; Santell et al., 1997). However, Levy et al. (1995) reported that following genistein treatment of rats during early pregnancy, the number of males was higher than females among the progenies although the sex ratios were not different that in control. Some flavonoids, such as chrysin, are natural aromatase inhibitors (Chen et al., 1997) and may be used to boost low levels of testosterone in aging males. Results of using tamoxifen, an aromatase inhibitor, in tilapia are controversial. Hines and Watts (1995) are the only authors to report a masculinizing effect of this compound (100 mg/kg of food) in a hybrid tilapia. In the Tenth Work Plan, we intend to evaluate the potential action of different phytochemicals, including genistein, quercetin, gossypol, and oleanolic glycoside on sex differentiation of tilapia. Our laboratory is able to isolate and quantify those phytochemicals in fish tissues using a high-performance liquid chromatography method.

Quantified Anticipated Benefits

The assessment of MT and MT metabolites persistence and fate in tilapia and water will provide information on the potential risks of using MT-feeding technology to produce all-male populations. The use of phytochemicals as an alternative method to produce monosex populations of tilapia will address human and environmental safety issues. Fish offered to the consumer will not be treated with MT and producers may have an alternative method for producing all-male populations of tilapia based on natural products, which do not require FDA approval.

Research Design

Location of Work: The feeding experiments will be performed both at the Laboratory of Aquaculture, Universidad Juárez Autónoma de Tabasco, Mexico, and at the School of Natural Resources, The Ohio State University, Columbus, Ohio. The experimental diets as well as RIA and HPLC analysis will be carried out at The Ohio State University. The Aquaculture laboratory at the Ohio State University has extensive experience on nutrition, reproduction and sex reversal in tilapia (Mbahinzireki, 2000). We continue to work on phytochemicals in fish diets and their metabolism (Lee et al., 2001; Dabrowski et al., 2001). Over the years, several experiments in our laboratory involved the use of MT and monitoring concentration of this chemical in fish tissues (Rinchard et al., 1999).

Methods

Determination of Methyltestosterone Metabolites Concentration in Tilapia Flesh and Water

The experiment will be conducted on first feeding of genetically all-female tilapia *Oreochromis niloticus*. All-female tilapia populations will be obtained by fertilization of normal eggs with sperm from a phenotypic male genotypically female (XX chromosome set) (Guigen et al., 1999). Fish will be assigned randomly into six tanks (300 fish/tank) and fed *ad libitum* (3 tanks/dietary treatment) for 30 days. MT will be dissolved in ethyl alcohol and incorporated into a commercial diet at a dose of 40 mg/kg (Abucay and Mair, 1997). Control food will also be treated with ethyl alcohol. Water quality will be monitored throughout the feeding experiment. Temperature and dissolved oxygen will be determined on a daily basis with biweekly measurements of total ammonia-nitrogen and pH. Every 10 days, fish and water will be sampled (n=15) from each tank and frozen for further analysis. At the end of the experiment, growth performance and survival will be evaluated. Sex ratio will be determined by microscopic analysis of gonadal squashes (Guerrero and Shelton, 1974; Guigen et al., 1999). At that stage ovaries are characterized by the presence of oocytes easily identifiable in their auxocytosis or previtellogenic stages, whereas testes are characterized by their typical lobular configuration (Nakamura and Nagahama, 1985). MT and MT metabolites in fish tissues and water will be determined using RIA (Rinchard et al., 1999) and HPLC (Williams et al., 2000) following extraction.

Evaluation of Potential Action of Phytochemicals on Sex Differentiation of Tilapia

The experiment will be conducted on first feeding genetically all-female tilapia *Oreochromis niloticus*. Fish will be randomly distributed into 21 aquaria at a density of 100 fish per aquarium with three replicates per treatment. One hundred larvae will be weighed and frozen for subsequent analysis of whole body composition. Fish will be fed at a restricted ration up to 90% satiation for 4-6 weeks. Semi-purified diets will be formulated based on our previous experience (Lee and

Dabrowski, 2001). Semi-purified diets will be used because they are least contaminated with natural steroids (Feist and Schreck, 1990). In order to determine the effects of phytochemicals on sex differentiation, seven casein-gelatin based diets will be prepared. Diet 1 (negative control) will be free of phytochemicals. Diets 2 and 3 (positive controls) will contain either MT at a dose of 40 mg/kg (Abucay and Mair, 1997) or ATD (1,4,6-androstratiene-3-17-dione; aromatase inhibitor) at an initial dose of 150 mg/kg (Guigen et al., 1999). Diets 4 to 7 will contain gossypol, genistein, quercetin and oleanolic glycoside at a dose of 500 mg/kg. The amounts correspond to the levels commonly found in seed meals of plants (Benneteau-Pelissero et al., 2001; Dabrowski and Lee, 2001). In the second year of study concentration of phytochemicals will be modified. Water quality will be monitored throughout the feeding experiment. Temperature and dissolved oxygen will be determined on a daily basis with biweekly measurements of total ammonia-nitrogen and pH. At the end of the feeding trial, growth performance will be evaluated in terms of final individual body weight, survival (%), specific growth rate (SGR, %) and weight gain (%). Fish from each dietary treatment also will be sampled for proximate analysis (water, protein, lipid, ash) and phytochemicals analysis (Dabrowski et al., 2001). Sex ratio will be determined as previously described (F.2.1).

Statistical Analysis: Analyses will be performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Data on growth performance and survival will be subjected to one-way analysis of variance (ANOVA) followed by a comparison of means using Scheffe's F test (Dagnelie, 1975). The Chi-square test will be used to determine alterations in sex ratios. Normality and homogeneity of variance tests will be performed on raw data. Sample distributions violating assumptions will be log-transformed before analysis. Data, expressed as percentages, will be arc sine-transformed before analysis. All differences will be regarded as significant at $P < 0.05$.

Regional Integration

Tabasco is considered among the States of Mexico with the highest potential for both intensive and extensive aquacultural development. Moreover, fish consumption constitutes an important part of the rural lifestyle in the State of Tabasco. Therefore, research efforts being proposed are logical initial steps toward developing sustainable aquaculture in the region. The research will benefit the entire region by providing pertinent information on masculinization of tilapia using natural phytochemicals.

Schedule

Determination of Methyltestosterone Metabolites Concentration in Tilapia and Water

July-August 2001, preparation of the experimental diets (MT and control);
September 2001, production of all-female tilapia population;
September-October 2001, feeding experiment and sampling;
November 2001-July 2002, measurement of MT and metabolites in tilapia and water using radioimmunoassay and high-performance liquid chromatography/mass spectrometry;
July-December 2002, data analysis and preparation of reports and publications.

Evaluation of Potential Action of Phytochemicals on Sex Differentiation of Tilapia

October 2001, formulation and preparation of the experimental diets
November 2001, production of all-female tilapia population;
December 2001-February 2002, first set of feeding experiments and sampling;
March-April 2002, measurement of phytochemicals in tilapia tissue;
May 2002, production of all-female tilapia population;
June-July 2002, second set of feeding experiments and sampling
August-September, measurement of phytochemicals in tilapia tissue;
October-December 2002, data analysis and preparation of reports and publications.

Final Report: 30 April 2003

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Selection of a New Nile Tilapia Genetic Line to Provide Broodstock for Southeastern Mexico

Reproduction Control Research 2 (10RCR2)/Experiment/Mexico

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Objectives

1) Evaluate the performance of wild and introduced stocks of Nile tilapia based on traditional genetic selection.
2) Provide tilapia fry farms in Southeastern Mexico with a good quality broodstock.
3) Provide farmers and institutions with a protocol for breeding strategies for the maintenance of the broodstock quality and for tilapia genetic enhancement.

Significance

Tilapia (*Oreochromis* spp.) culture has been the principal aquacultural activity in Southeastern Mexico since the 1960s – 1970s (Fitzsimmons, 2000). With the introduction of tilapias to Mexico, the different institutions responsible of fish production created remarkable expectations among Mexican farmers and investors. Unfortunately, several problems have resulted in these expectations being unrealized. The loss of the lineages introduced, the lack of effective genetic selection programs, and poor management decisions have created disappointment and uncertainty around tilapia culture in Mexico. Meanwhile, other countries in Central and South America have demonstrated that tilapia culture can be a remarkable investment for both business and social projects.

Because of its geographic and hydrological components, the Southeastern region of Mexico has been considered as one of the top areas in Mexico for aquaculture and especially for tilapia culture. Currently there are 6 main tilapia hatcheries in the Mexican states of Tabasco and Chiapas that provide fry to more than 5,000 small tilapia farmers. However, the quality and quantity of the fry provided has been a constant criticism from the farmers to the hatchery managers. In 1993, Contreras-Sánchez and Fernández documented in an internal report for the State's Office for Development (Secretaría del Desarrollo) the need for establishing a new tilapia line appropriate for meeting the needs and conditions of the Central American tropics. This document also suggested the need to establish broodstock selection programs and the formation of high quality broodstock lots in the fry production facilities. To date, all hatcheries in Tabasco and Chiapas are buying fry from the Mexican States of Veracruz, Oaxaca, and Guerrero to partially fulfill the demand. However, the quality of the fry is still low. We propose to develop a program for acquiring local and foreign tilapia broodstocks, conduct a selection program following Bhujel's (2000) recommendations for broodstock management strategies, and create a broodstock selection program that avoids risk by following elements in a risk assessment analysis for determining the genetic vulnerability of the broodstock outlined by Currens and Busack (1995).

We have identified a stock of wild Nile tilapia in the Usumacinta River that shows good phenotypic traits (small head, small tail, large body, and uniform color). In the present study we proposed to select a group of adult fish that will be used for progeny testing and traditional genetic selection. Another group of fish from a region that shares similar climatic conditions with Tabasco will be obtained from a tilapia-breeding center (ideally GIFT tilapia from the Philippines) and both local and introduced lines will be managed under the same breeding protocol. The quality of the fry of both stocks will be contrasted (condition factor, growth, conversion factor) and traditional selection will be used for genetic enhancement. Quality of the fry produced will be estimated using growth, fertility, survival, and feed utilization as indicators.

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 improve significantly the production of good quality fingerlings and have a favorable impact on more than 5,000 subsistence farmers and medium-scale producers.

Extension workshops and outreach activities and materials that help increase the awareness of farmers and producers about the importance of maintaining broodstock quality over time will be prepared and offered through a variety of selected public information and training institutions.

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 population of extremely poor campesinos characterizes the rural social structure.

Research Design

Site: Acclimation of adult fish and progeny testing will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, México.

Activities: Capture of wild adult Nile tilapia from Jonuta, Tabasco; acquisition of GIFT tilapia from the Philippines; acclimation of fish to laboratory conditions; genetic selection using phenotypic traits

Laboratory and Pond Facility: Universidad Juárez Autónoma de Tabasco (DACB); 20 aquaria (50 l), 3 reproduction concrete tanks (8 m^2), 50 net cages (1 m^3) for grow-out, 3 grow-out ponds (200 m^3), a total of 10 females and 30 males for production of fry. Universidad Juárez Autónoma de Tabasco (DACA); 2 reproduction concrete ponds (50 m^2), 2 grow-out ponds (50 m^2). 20 net cages (1 m^3) for grow-out.

Stocking Density for Spawning: 3 females:1 male/m^2

Stocking Density for Grow-Out: 100 fish/m^2 (first 2 months); 50 fish/m^2 (2 months); 20 fish/m^2 (last 2 months)

Culture Period: 6 months (three cycles)

Test Species: Nile tilapia (*Oreochromis niloticus)*

Nutrient Inputs: None

Water Management: Flow through 10% water exchange/day

Sampling Schedule: Total length, fork length, weight, total height, total width, and head and tail size of the tilapias will be measured once a month.

Statistical Methods and Hypothesis: $H_0$1: Fish with the highest condition factors show no decrease in food conversion. To select the most valuable traits, multifactor anovas and discriminant analysis will be performed using the morphometric factors as the independent variables and food conversion rate as the discriminant factor. Fish with the highest condition factor will be selected using Multivariate Analysis.

Schedule

Data collection, 7/01 to 2/03; Technical report, 3/30/03

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IGF as a Growth Rate Indicator in *Oreochromis niloticus*

Reproduction Control Research 3 (10RCR3)/Experiment/Philippines

Collaborating Institutions
Central Luzon State University
Remedios B. Bolivar

Florida International University
Christopher L. Brown

Objectives

1) Overall: Test the sensitivity and accuracy of the growth mediator, Insulin-like Growth Factor (IGF-1) as an indicator of growth rate in *Oreochromis niloticus.*
2) Clone the IGF-1 gene in *Oreochromis niloticus*.
3) Develop an RNAse protection assay for IGF-1 in *O. niloticus.*
4) Adapt and test available methods for determining RNA and DNA content and RNA/DNA ratios in *O. niloticus* tissues.
5) Use the methods specified in objectives 4 and 5, above, to test the responsiveness of cellular indicators of growth in young *O. niloticus*, exposed to nutritional variables.

Significance

Faculty development has been a high priority at Central Luzon State University, and a steadily increasing percentage of faculty at CLSU hold the Ph.D. The Freshwater Aquaculture Center is the highest-ranking academic unit in this regard within CLSU (R. Undan, CLSU President, pers. comm.). Many of the FAC faculty have earned their Ph.D. from US and Canadian universities, and a concerted effort is being made to advance the higher educations of additional faculty. Clearly, the establishment of a high standard of education has contributed to the increasing academic strength of the FAC, and the desire to continue to develop faculty research capabilities is a goal of the CLSU administration.

In order to maintain the necessary numbers of faculty needed to sustain research programs and curriculum offerings, it is usually the case that one faculty member is allowed a Leave of Absence to pursue doctoral studies at a time. Emmanuel Vera Cruz is considered to be one an outstanding young faculty member at CLSU, and he has applied for admission to the Doctoral Program at Florida International University in the Department of Biological Science. He graduated summa cum laude, and is eager to pursue a rigorous doctoral program in fish physiology and genetics. Initiating that program is one goal of this educational development activity.

The proposed activity will include a program of doctoral research designed to add a potentially valuable dimension to the ongoing studies at CLSU on the subject of feeds and fertilizers. Specifically, we have found in large-scale farm trials that either delayed feeding or feeding a sub-satiation-level diet benefit are realized in the cost savings that occurs as a result of the reduction in the amount of processed feed that must be purchased by farmers. Our analysis, based on trials at seven participating commercial farms in the Philippines, showed that delayed feeding could result in a lowering of feed costs by approximately 37% without a significant loss of crop value at harvest (Brown et al., 2000).

One factor limiting the number of experiments that can be carried out on the subject of diet optimization is the extended length of time that is required to see results. We presently stock juvenile fish and sample throughout a full grow-out period, which in the Philippines, typically takes about five months. The research goal in the planned doctoral program will be to evaluate and validate short-term physiological indicators of growth. Both RNA/DNA ratios and the quantification of circulating Insulin-like Growth Factor (IGF-1) will be used for this purpose.

Quantified Anticipated Benefits

The proposed research will provide highly specific cellular and molecular tools that can be used for rapid evaluation of diet and other culture parameters. With the capability to monitor the proximal regulatory mechanisms involved in growth, it will be possible to determine optimal grow-out conditions without having to wait for a complete growth cycle to be completed. These methods should be capable of providing a clear indication of growth stimulation within a few days of exposure to experimental conditions.

We have found already that it is possible to reduce grow-out costs by providing reduced rations, and other means of improving grow-out efficiency are very likely. Presently, the effectiveness of locally produced feeds applied judiciously to reduce farmers' bills must be evaluated in large-scale farm trials. It should be possible to sort through and evaluate the various feeding and environmental variables much more efficiently with the help of short-term indicators of growth, such as the circulating level of IGF-1 or the RNA/DNA ratio. Conceivably, this approach would allow the effectiveness of a dietary or environmental factor or combination of factors to be determined in a matter of days, rather than months.

The scientific benefits and impacts of an educational development activity must ultimately be viewed in terms of the long-term institutional and humanitarian effects. Solid progress toward the Ph.D. will be one such quantifiable benefit. At present the Freshwater Aquaculture Center does not have anyone with the expertise to lead a project in DNA technology, although the interest is certainly there among students and faculty. The proposed project will involve the cloning of a portion of the tilapia genome (the IGF-1 gene) and its detection using standard molecular methods. These techniques will be propagated at the host country institution, allowing CLSU to be a participant rather than an observer in the development of new applications of biotechnology. Documented mastery and specific accomplishments in the use of these techniques will be other quantifiable benefit (number of abstracts produced, successful cloning, etc.).

This approach is in keeping with the spirit of the recent PD/A CRSP white-paper policy document on biotechnology and genetics (Brown and Bart, 2000), which advocated for the development of non-invasive applications of DNA technology. The use of a regulatory compound as a marker or indicator of growth is non-invasive; that is, it does not involve reconstruction or other potentially controversial manipulation of the genome. This is, nevertheless, a potentially valuable technology. The cost of developing a new strain of fish, for example, can be dramatically reduced if the rapidly growing individuals can be identified within a few days using DNA markers, rather than a few months by the conventional feed and weigh approach.

Research Design

Location of Work: Mr. Vera Cruz will travel from CLSU in Nueva Ecija to carry out his doctoral studies at the Biscayne Bay campus of Florida International University, North Miami Beach, Florida. He will be enrolled as a full-time doctoral student, using the typical configuration of doctoral candidates of 50% time in classroom study activities and 50% time as a Research Assistant. Research Assistantships are the usual arrangement for graduate student support.

A very well-equipped laboratory is presently available for these studies, and a new marine lab building is in the late planning stages, and will probably be available before the doctoral program is completed.

Methods: Insulin-like Growth Factor, IGF-I is the hormone accepted as directly responsible for promoting growth in fish, as in other vertebrates. The actions of growth hormone and other anabolic agents are transduced through IGF-1, which is responsible for direct stimulation of growth in muscle and other tissues. For this reason, the detection of IGF-1 represents one of the best conceivable indications of growth at the proximal end of the process.

Not surprisingly, circulating IGF-I levels correlate well with growth rates in animals (Jones and Clemmons, 1995; Beckman et al., 1998). The relationship of IGF-I levels and growth rate is more consistent than that of growth hormone (GH) with growth rate. Growth hormone levels can become dissociated with growth rate under some conditions in which the correlation of IGF-I with growth persists (Duan et al., 1997). For these reasons, the detection of IGF-1 is gaining wide acceptance as one of the most accurate and direct short-term indicators of growth rate available (Fruchtman et al., 1997).

Our colleagues at North Carolina State University have been working with the cloning of IGF genes in the striped bass, and have agreed to provide technical and collaborative assistance as necessary for this project. A letter confirming collaboration from Dr. R. Borski is attached. The detection of IGF-I in Nile tilapia will require the cloning of the IGF-1 gene in *O. niloticus*, and the adaptation of established methods based on a highly sensitive RNAse protection assay previously developed in rats (Borski et al., 1996; Borski et al., 2000). Primers are available which should make this a straight-forward task.

A second approach will also be used in the assessment of cellular indicators of growth rate. Because cellular DNA content remains at baseline as RNA concentrations rise with the rate of cellular protein synthesis, the ratio of RNA to DNA concentrations has often been used as an index of protein synthesis, which reflects changes in growth rate. RNA/DNA data support this association (Bulow, 1987), although the sensitivity of RNA/DNA ratios as an indicator is limited.

Our plan is to develop the tools needed to test and apply both short-term indicators of growth rate in *O. niloticus.* This approach will dramatically reduce the long and costly production trials required for growth selection inherent to traditional breeding programs. Using the data already obtained in our continuing farm trials, and the data expected to be obtained in the course of Work Plan 10, we will test the levels of these biomarkers in laboratory-reared fish on similar diets as compared with the growth results obtained in the farm trials. This will serve either to validate or refute the efficacy of these tools as predictors of growth rates. The use of both RNA/DNA ratio and heterologous IGF-1 taken in combination may have a more accurate predictive value than either indicator used alone (R. Borski, pers. comm.).

One limitation on the proposed program of study is the use of a genetically selected strain of tilapia in the farm trials (the GIFT Foundation fish) that are unavailable in Florida. Ideally, the correlative studies seeking relationships of growth data in farm trials and biomarkers for growth in laboratory tests should use identical fish. Recognizing this shortcoming, we still believe that the proposed work will be valid, since it will be carried out in the same species. Eventually it is anticipated that the cellular methods we adapt to *O. niloticus* in the course of this project will be applied *in situ* (in the Philippines) and elsewhere.

Regional Integration

The enormity of the value of Nile tilapia worldwide provides strong incentive to test and validate cellular tools that can be applied in its selective breeding and domestication. The regional integration of this technology is a long-term prospect, which will be completed after Mr. Vera Cruz has completed his studies and returned to his teaching and research position at CLSU. His technical knowledge and experience will continue to grow, as will his influence as he serves as mentor for students throughout his chosen career as a scholar and educator.

We will post and regularly update research progress on the project website, described in more detail under the Farm Trials investigation. Interest in and frequency of downloads of research products (abstracts, manuscripts, etc.) derived in the course of this educational development activity will be monitored.

Schedule

Year 1

Date	Activities
April 2001 (pre-project)	Complete application file for FIU Doctoral program Planned visit of Mr. Vera Cruz and Mr. Eddie-Boy Jimenez to FIU under Ninth Work Plan activity
9/30-11/31/01	1) Test and develop RNA/DNA methods 2) Clone *O. niloticus* IGF-1 gene
11/15-12/15/01	Initiate feeding experiments beginning with sub-satiation feeding, with biomarker endpoints
12/1/01	1) Enroll Mr. Vera Cruz in doctoral program 2) Planned move from Muñoz to Miami 3) Begin setting up experimental culture system and obtaining fish stocks

Year 2

Date	Activities
7/1-8/01/02	Assemble Doctoral committee, complete formal Doctoral Proposal
9/1-9/30/02	Initiate feeding experiments using alternate day (Tenth Work Plan) strategy
11/02	Report efficacy of biomarker studies
4/10/03	Report results at project workshop at CLSU farmers
4/30/03	Submit final project report

Note: the typical duration of a doctoral program exceeds the length of time available in the Tenth Work Plan, which is less than two years. We are committed to seeking additional sources of funding, assistantships, etc. during the portion of the program of study that occurs after the completion of this activity in order to see this degree program through to completion.

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Development of a Trophic Box Model to Assess Potential of Ecologically Sound Management for Cove Aquaculture Systems in Tri An Reservoir, Vietnam

Aquaculture System Modeling Research (10ASMR1)/Study/Thailand and Vietnam

Collaborating Institutions
Asian Institute of Technology, Thailand
C. Kwei Lin
Yang Yi

University of Agriculture and Forestry, Vietnam
Le Thanh Hung

University of Michigan
James S. Diana

Objectives
1) Determine biomass production of various trophic levels in the fish culture cove.
2) Construct a trophic box model for the selected cove.
3) Recommend the ecologically sound stocking and management strategies for cove aquaculture.

Significance
A large number of reservoirs have been built in Indochina, mostly for irrigation, electrical generation and domestic water supply. With few exceptions, the fishery of these reservoirs also provides an important source of animal protein and livelihood for people living around the reservoirs. However, most reservoirs in the region are relatively unproductive with catch ranging from <10 to 65 kg ha^{-1} yr^{-1} (Anon., 1998). In Vietnam, many reservoirs exist in the central highland and some are used for fish production through enhancement stocking and cage culture. In large reservoirs, catch per unit effort of wild fisheries is quite low due to low productivity of pelagic water. Cage culture in large reservoirs often suffers from heavy mortality and usually requires protein-rich feed, such as small fish caught in the reservoir itself. An alternative means to enhance fish production would be to pen semi-enclosed shoreline areas with barrier nets between the main reservoir. Such a system is termed cove culture. Dendroid coves are a prominent feature of most mountain reservoirs. The ideal cove for aquaculture is one that contains mainly a littoral zone of 1-2 m depth with alternating flood and exposure during rainy and dry seasons. Cove culture has been widely used in Chinese reservoirs (Li and Xu, 1995). Some advantages of cove culture are ease of access from shoreline (compared to pelagic cage culture), more available food sources in the littoral zone, ease of harvest during the dry season, and low cost.

Tri An Reservoir, located 75 km from Ho Chi Minh City contains 50 coves of various size in its 324 km^2 surface area. At least a dozen cove culture systems already exist in this area. However, fisheries development of Tri An Reservoir is threatened by water pollution from runoff, organic matter input from surrounding vegetation and wastes from cage culture (Luu, 1998). Development of cove culture relying on natural foods within the reservoir is ecologically sound and most likely more sustainable.

The most common local method of cove culture stocks mainly herbivorous and detritivorous species such as common carp *Cyprinus carpio*, bighead carp *Aristichthys nobilis*, tilapia *Oreochromis niloticus*, and grass carp *Ctenopharyngodon idella*. Stocking density and species ratios are based on those practiced for pond culture (Yang et al., 1990). As Tri An Reservoir is rich in benthic invertebrates and small fishes (such as Clupeids and freshwater shrimp), stocking of carnivorous species with high food value, such as sand goby *Oxyeleotris marmoratus* and snakehead *Channa striata*, could enhance production and economic gain from cove culture. Many efforts have been made to catch sand goby fingerlings from the reservoir for cage culture, but all failed because of mass mortality after moving sand goby to cages. An alternative method may be to culture sand goby in coves.

So far, little information is available on the natural food productivity of a cove. Such information could enable us to estimate the productivity of natural food at various trophic levels, predict carry capacity of stocked fish species of different trophic levels, and thus determine the quantity and combination of fish to be stocked based on food availability. An ecosystem model could be constructed to assess trophic status for a cove. An average system can be described with a steady-state model (Christensen and Pauly, 1993). Such static models can give important information on energy flow and biomass storage, which should be measured to validate such a model. However, coves within a reservoir are often not in a steady state, so that information gained from such an analysis is most likely at best an approximation of the fishery potential of a cove.

Anticipated Benefits

The results of this study could be used to enhance fish production with greater economic return from cove culture. The study will also establish a case of sound management of fish culture in reservoirs that is more ecologically sustainable than current cage culture. The development of realistic ecological models would provide a new strategy for development and management of reservoir systems, and would facilitate extension of cove culture systems throughout the region. Use of Ecopath and a steady state analysis is only the first step in the development of realistic models, but is a necessary first step.

Research Design

Location: Truong Dang (TD) Aquaculture Cove, Tri An Reservoir, Vietnam; lab work at Aquaculture Laboratory, University of Agriculture and Forestry, Vietnam

Methods: Construct a trophic box model for TD Cove using the ECOPATH 3.0 approach and software, developed by ICLARM (Dalsgaard and Oficial, 1998). The model will be made on a dry weight (DW, g) per area basis.

Parameters to Be Measured for Constructing the Model

- Forcing functions: area and volume of the cove, input of terrestrial plants, pH, dissolved oxygen (DO), water temperature and transparency
- State variables: the biomass (gDW/m^2) of detritus, phytoplankton, zooplankton, benthos, and cultured fishes
- Area and volume of the cove: calculated by using the SURFER 6.0 software for Windows from a 1-m depth contour map with ground truth measurements in June 2002 (Keckler, 1997)
- Biomass of terrestrial plants: measured by randomly collecting plants from at least ten 1 m^2 plots per time, drying at 103°C and weighing (Winberg, 1971)
- pH, DO, alkalinity, water temperature and transparency: measured by using standard CRSP methods
- Biomass of detritus: estimated by measuring organic matter concentration for 10 collections per time from bottom sediment (5 cm top layer) (Boyd, 1995);
- Biomass of phytoplankton: estimated by measuring concentrations of chlorophyll *a* from ten water collections per time using standard CRSP methods;
- Biomass of zooplankton: calculate the average dry weight of the population from an estimate of average length, by using length-dry weight regression equations. Again, 10 collections will be made per time (Dumont et al., 1975 cited by Bernardi, 1984);
- Biomass of benthos: separate benthos from sediment sample (f.2.2.4) with a soil sieve at 500 mm mesh size, then dry at 103°C or 550°C and weigh (Lin, 1974);
- Biomass of different cultured fish groups: were calculated as the mean of the biomass at stocking and at harvest (Ruddle and Christensen, 1993).

Sampling Schedule for Above Parameters

Sampling period: 8 months from August 2002 to February 2003.

Water quality (pH, DO, water transparency, and temperature) and biomass (detritus, phytoplankton, zooplankton, and benthos): measured for four distinct periods in August, October, December 2002 and February 2003. Each period has three sampling times in two weeks.

Biomass of terrestrial plants: measured for two distinct periods in June and July 2002. Each period has three sampling times in two weeks.
Biomass of different cultured fish groups: are assessed at stocking and at harvest time.

Regional Integration

In many Asian countries, reservoirs have been developed for fish production, making a significant contribution to overall inland fish production. However, the production is usually low and faces increasing fishing pressure. Development of cove culture will enhance fish production and provide an alternative strategy for nature-based aquaculture in reservoirs in the region.

Schedule

June 2002 to February 2003
Report submission: April 2003

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Amazon Aquaculture Outreach

New Aquaculture Systems/New Species Research (10NSR1)/Activity and Study/Peru

Collaborating Institutions
Instituto de Investigaciones de la Amazonia Peruana, Peru
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Susan T. Kohler

Objectives

1) Provide extension services to the local community to promote sustainable aquaculture in the Peruvian Amazon.
2) Conduct demonstration projects with local fish farmers to expose them to new species and/or techniques.
3) Provide short courses to governmental and NGO personnel to develop a network of aquaculture extensionists in Peru and neighboring countries.
4) Establish a specialized website on Amazonian aquaculture and species to provide for information exchange and networking.

Significance

Aquaculture has been practiced for the last ten years in areas along the only road stretching from Iquitos to the City of Nauta (95 km away). Total pond surface hectares have increased from 22 in 1991 to slightly above 100 in 2000 (Alcántara, 2001). These fish farmers received support from public and private entities. Still the industry's rate of development was minimal and results were scarce. Until recently, poor technology transfer and misinformation to the farmers led to poor production. Furthermore, some of these extension programs dissolved, leaving farmers stranded and feeling uncertain of the benefits of aquaculture. Still, a sociological study determined that over 80% of the human population along the Iquitos-Nauta road and in the Tamishiyacu and Mazan River areas had great interest in aquaculture practices, and over 80% of practicing farmers saw aquaculture as more feasible than other forms of land farming (Molnar et al., 2000).

It has been only in the past two years that fish farmers have been properly supported with aquaculture extension activities. The food security program (PROSEAL) directed by Terra Nuova (NGO) and IIAP has accounted for most of this enhancement. Their goal has been to promote the organization of fish farmers into self-sustainable associations in order to develop the aquaculture industry in a coordinated form, allowing for vital farmer interactions and common education among them. In the past two years, PROSEAL has received support from IIAP, Terra Nuova, PD/A CRSP, Maynas municipal government, Ministry of Fisheries, as well as additional contributions from two other NGOs. PROSEAL goals have been met by developing continuous workshops aimed at teaching local fish farmers about the production process, ranging from pond construction and pond management to commercialization of their product. All activities are directed at native fish species.

By the end of January 2001, PROSEAL has greatly impacted the aquaculture industry in the Iquitos region. They now provide services to 88% of fish farmers who account for almost 55% of total fish ponds in the region (Table 1). PROSEAL has been a direct beneficiary from the CRSP program in Peru. Results from research conducted at our host country facilities provided much of the information that PROSEAL extended to farmers. Thanks to leadership provided by our host country PI, Dr. Fernando Alcántara, as well as other IIAP and UNAP members, valuable information developed from our project has been transferred to the local area fish farmers via PROSEAL.

Table 1. Local organizations and their extension responsibilities along the Iquitos-Nauta road as of January 2001 (Alcántara, 2001).

Organization	Surface area (ha)	%	Fish farmers	%	Ponds	%
PROSEAL	25.50	24	182	88.26	230	54.89
CURMI (NGO)	8.99	8	41	4.46	42	10.02
CARITAS (NGO)	10.95	10	10	1.09	10	2.39
FONDEPES (GO)	1.80	2	6	0.65	6	1.43
Independent farmers	52.32	49	49	5.33	84	20.05
Government farms	6.25	6	2	0.22	47	11.22
TOTAL	105.81	100	290	100	419	100

Efforts began in the Ninth Work Plan to create an extension work committee, which will allow us to formally integrate our extension activities for the Tenth Work Plan into the existing host country program. The committee will ensure proper cooperation among all participating entities, and help avoid redundancy in the proposed work region. Alcantara, with PD/A CRSP support, will continue to serve as the lead PROSEAL aquaculture extensionist. The PROSEAL project is scheduled to terminate in December 2001. Accordingly, the continuity of this important effort will be reliant on PD/A CRSP support.

Quantified Anticipated Benefits

The development of sustainable aquaculture will benefit many sectors throughout the Peruvian Amazon. Rural farmers will benefit from the addition of an alternative form of agriculture. Aquaculture production requires considerably less land than that needed for cattle ranching. Moreover, ponds can be used year-after-year whereas rain forest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons. Such lands, once abandoned, usually can no longer support normal jungle growth. Both rural and urban poor will benefit by the addition of a steady supply of high quality protein in the marketplace. Aquaculture of *Colossoma* and/or *Piaractus* should relieve some of the fishing pressure on these overharvested, native species. These species have been suggested to play a crucial ecological role in disseminating seeds from the flooded forest (Goulding, 1980; Araujo-Lima and Goulding, 1997). Accordingly, the aquaculture of *Colossoma* and/or *Piaractus* may be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Peruvian Amazon. Host country consumers and fish farmers, researchers, extensionists and planners, local and foreign Latin-American governmental organizations and/or NGOs and users of global CRSP-sponsored models and data will benefit from this activity. Development of a Latin American network of Amazonian species producers and researchers could catalyze regional efforts to fortify the growing industry and to explore new aquaculture candidates to diversify production.

Activity Plan

Objective 1: Provide Extension Services to the Local Community to Promote Sustainable Aquaculture in the Peruvian Amazon

We will continue to reinforce extension activities with the 240 local farmers currently being served along the road system between the cities of Iquitos and Nauta, and expand the coverage to at least one other community near to Iquitos. Our activities will reinforce and complement similar activities being conducted by the Italian NGO Terra Nuova. Farmers will be provided with knowledge gleaned from the CRSP-sponsored studies with *Colossoma* and *Piaractus* conducted in the Eighth and Ninth Work Plans. Accordingly, we will:

Provide workshops to existing and prospective fish farmers in the Iquitos region. Specifically, we will compile a Spanish-language production manual for *Colossoma* and *Piaractus* 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 for teaching prospective farmers the basics for pond culture. A video displaying standard practices for spawning and culturing *Colossoma* and *Piaractus* will also be produced to complement the written manuals. At least two workshops will be provided each year of the Tenth Work Plan, and all farmers currently producing fish will be invited. At least one workshop per year will be provided to prospective fish farmers in the region. These farmers will be identified from the many inquiries made to IIAP for general information on fish farming. The latter workshops will be more general in nature and will serve as a primer for the more advanced workshops provided to existing producers. All workshops will include orientation on the business aspects of aquaculture.

Provide aquaculture advisement via site visits to local farmers. We will make bi-monthly site visits to fish farms in the Iquitos area. Farms will be visited on a rotational basis so that every farm is visited at least once each quarter. Farmers will be provided with information on fish husbandry and pond maintenance, as well as with any new developments learned through our research activities. Standard water quality parameters (temperature, dissolved oxygen, pH, hardness, alkalinity, carbon dioxide, total ammonia nitrogen, and nitrite) will be measured at representative farms throughout the region.

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.

<u>Objective 2: Conduct Demonstration Projects with Local Fish Farmers to Expose Them to New Species and/or Techniques</u>

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

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

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

The second technique will analyze the feasibility of transferring technology to area fish farmers by conducting on-farm research and demonstration projects. To ascertain the effectiveness of this model, a preliminary density study with *Arapaima gigas* has been designed and will be carried out at three local fish farms over an eight-month period. Two, four and six fish/m^3 will be stocked in cages. The 3 x 3 x 1.2 m floating cages will be constructed with 0.25" plastic mesh and 2" PVC pipes for the frame, and balsa-wood buoys for flotation. The food source will be biological silage of discarded commercial fish and nuisance invaders at the IIAP research ponds. This study will be conducted by two UNAP undergraduate students as a component of their thesis work.

<u>Objective 3: Provide Short Courses to Governmental and NGO Personnel to Develop a Network of Aquaculture Extensionists in Peru and Neighboring Countries</u>

Two intensive training courses for small groups of governmental and nongovernmental personnel conducting aquaculture research and/or extension activities in the Amazon Basin will be offered yearly at IIAP, Iquitos, Peru. For each course, five qualified Latin-American participants will be invited to participate. The first course will be offered to train aquaculturists in extension techniques. Extension techniques practiced successfully by IIAP and Terra Nuova will be emphasized. This course will consist of Three days (eight hours per day) of lecture and one full day of practical field work. The second course will be directed to extension personnel with little or no training in aquaculture. Extension personnel will learn broodstock selection and handling, spawning techniques, incubation, larviculture, grow out, disease prevention, all specifically related to the species of *Colossoma* and *Piaractus*. This intensive module will also cover three days (eight hours per day) of lectures and one day with practical laboratory work to teach hormone injection, spawning, fertilization, incubation and larviculture techniques.

Objective 4: Establish a Specialized Website on Amazonian Aquaculture and Species to Provide for Information Exchange and Networking

A website on Amazonian aquaculture and species will be designed to allow for information exchange and networking. The website 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, FAO, World Wildlife Fund, etc. A chat room 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 list-serve will be established and maintained for the purpose of relaying relevant information on Amazonian aquaculture and species. The number of hits to the site will be enumerated to determine the site's exposure.

Regional Integration

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

Schedule

All activities will take place from 1 July 2001 through 30 April 2003. A final report will be submitted on or before 30 April 2003.

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Studies on Reproduction and Larval Rearing of Amazonian Fish

New Aquaculture Systems/New Species Research 2 and 2A (10NSR2 and 2A)/Experiment/Peru

Collaborating Institutions
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Objectives

1) Evaluate and compare growth performance of gamitana, *Colossoma macropomum*, and paco, *Piaractus brachypomus*, larvae fed different feeds.
2) Determine changes in plasma sex steroid hormones during an annual cycle and those preceding ovulation and spermiation in two Amazonian catfishes, *Pseudoplatystoma fasciatum* and *P. tigrinum.*

Significance

Gamitana, *Colossoma macropomum*, and paco, *Piaractus brachypomus*, are two important aquaculture species in South America (Saint-Paul, 1992). These Characidae have a commercial value because of their high growth rate and the quality of their flesh (Saint-Paul, 1992; Vieira and Johnston, 1996). During the Ninth Work Plan, we successfully induced ovulation and spermiation of *P. brachypomus* using LHRHa. Both genders were injected with two doses of LHRHa. The concentration of preparation was 0.0042 mg of equivalents of active hormone per ml. Males and females were injected with 1 ml/kg and 2.6 ml/kg, respectively. The priming dose (50% and 10% in males and females, respectively) was administrated in the morning, whereas the resolving dose (50% and 90% in males and females, respectively) was injected at 2200 h. Oviposition was observed within 8 to 16 hours following the resolving dose of the hormone and survival at 13 hours of incubation amounted to 68.5 ± 25%. Our challenge now is to rear these larvae in intensive culture using dry food. This is important because survival of larval paco stocked directly to ponds was very low in IIAPs experience. The information on the first feeding of Characidae is scarce. Recently, a feeding experiment was carried out to determine the relationship between the live *Artemia* feeding levels and growth rate in pre-weaning *C. macropomum* larvae (Sevilla and Gunther, 2000). However, Canzi et al. (1992) and Yamanaka (1988, in Canzi et al., 1992) reported that artificial diets are readily accepted by *P. mesopotamicus*. Therefore, we propose to investigate the potential for first feeding *C. macropomum* and *P. brachypomus* using commercial and experimental diets. Preliminary experiments in 2000 carried out by the authors confirmed the acceptance of formulated diets and growth of paco larvae.

The second objective of this study is focused on the controlled reproduction of two catfish species *Pseudoplatystoma fasciatum* and *P. tigrinum*, which are of interest as new aquaculture species in South America (Kossowski, 1996). In Peru, spawning of both species occurs in February-March (Alcantara, personal communication). In *P. fasciatum,* the oocyte size was used to evaluate the maturity of the gonads and a diameter of 1.8 mm indicated the readiness of the gonads (Kossowski, 1996). Final maturation and ovulation was achieved in several catfish species from South America using carp pituitary extracts or pituitary hormones (Cardoso et al., 1995; Kossowski, 1996). However, to the best of our knowledge, no information is available on the profiles of plasma sex steroids in both target species and we could possibly use this information to synchronize ovulation/spermiation in these fish (Dabrowski et al., 1996). The annual changes in the blood plasma steroids as well as the surge preceding the spermiation and ovulation (maturational hormones) can contribute to a better understanding of the dynamics of gonadal steroidogenesis. Moreover, such information will be useful in the development and standardization of breeding techniques through the use of natural and/or synthetic hormones. Preliminary data indicated that the level of estradiol-17α and testosterone in females of *P. fasciatum* raised in a pond at IIAP in March averaged 0.35 ± 0.2 ng/ml and 3.18 ± 2.5 ng/ml (n = 4).

Quantified Anticipated Benefits

The proposed study aims to investigate key aspects of nutrition and reproduction biology of several Amazonian freshwater fish species such as *C. macropomum, P. brachypomus, P. fasciatum*, and *P. tigrinum* in order to improve or develop sustainable aquaculture technology for these species.

Through our collaborative effort with Peruvian investigators we will be able (1) to develop the procedures of first-feeding of *C. macropomum* and *P. brachypomus*, and (2) to monitor and understand the dynamics of gonadal steroidogenesis during maturation of *P. fasciatum* and *P. tigrinum*. These data on steroid profiles will be correlated with the quality of gametes produced. Therefore, the first beneficiaries of this research will be the local producers of *Colossoma* and *Piaractus* species in the Peruvian Amazon. In our experience, larval gamitana and paco frequently experience low water levels in nursery ponds and high water temperatures. Development of the technology of intensive growth of these species and stocking 4–6-week-old juveniles will dramatically increase their survival and efficiency of production.

Catfish could be cultured in mono- or polyculture systems in order to control the native cichlids like *Cichlassoma* and *Aequidens*. Both species reach large sizes (20-40 kg) and have a wide distribution throughout the Amazonian basin in South America (Colombia, Venezuela, Brazil), so experiences 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 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 IIAP staff on various aspects of fish nutrition and reproduction.

Research Design

Location of Work: The experiments will be accomplished at the field station of the Instituto de Investigaciones de la Amazonia Peruana. Additional experiments with the experimental diets and radioimmunoassay analysis will be carried out at the School of Natural Resources, The Ohio State University, Columbus, Ohio, USA.

Methods

Objective 1: Growth Performance of Gamitana *C. macropomum* and Paco *P. brachypomus* Larvae

Larvae of both species obtained from induced reproduction will be used for the feeding experiment. The feeding trial will be conducted in a flow through system consisting of 12 cylindrical tanks (3 tanks/dietary treatment) supplied with aeration. Water quality will be monitored throughout the larval rearing process. Temperature (26–28°C) and dissolved oxygen (5-6 mg/L) will be determined on a daily basis with biweekly measurements of total ammonia-nitrogen and pH. Two days after yolk resorption, larvae will be randomly distributed at a density of 5,000 larvae/tank and fed at a restricted ration up to 90% satiation for 2-4 weeks. At the beginning of the experiment, 30 larvae will be weighed and frozen for subsequent analysis of whole body composition. Larvae will be fed 4 diets: 1) live food (*Artemia* nauplii), 2) a commercial diet (Kyowa Hakko Kogyo Co., Ltd., Japan), 3) an experimental casein-gelatin–based diet (Lee et al., 2001) and 4) an experimental lecithin based diet (Infante and Cahu, 1999). Both experimental diets will be formulated based on our previous experience and will be isonitrogenous (protein requirement: 40-55% for most larval fish, Dabrowski, 1986). Larval samples (n = 20) will be taken every week from each tank and fixed in buffered formalin for biometric measurements. At the end of the experiment, growth performance will be evaluated in terms of final individual body weight, survival (%), specific growth rate (SGR, %) and weight gain (%). Fish from each dietary treatment also will be sampled for proximate analysis (water, protein, lipid, ash).

Objective 2: Changes in Plasma Sex Steroid Hormones in *P. fasciatum* and *P. tigrinum*
Broodstock fish from both species will be raised in ponds at the IIAP. Water temperature and dissolved oxygen will be recorded daily. At the beginning of the gonad recrudescence, twenty-five fish of both species will be captured, individually measured, weighed and tagged (PIT-tags, Biosonic, Seattle, WA). Fish will then be released into their respective ponds. Blood will be collected every 3-6 months from the caudal vessel of unanesthetized fish using a heparinized syringe. Blood will centrifuged at 1,500 g for 15 min and the plasma stored at –20°C until assays. At the time of maturation (normal spawning time in Peru is late February to early March), spermiating males and robust females will be selected. Ovarian maturity will be assessed with a microscope using oocytes collected from the ovary with a catheter. Pairs of catfish will be moved into indoor 0.75-m^3 concrete tanks. In each tank, the male will be separated from the female by a net. Intensive aeration of the water will be provided upon fish arrival. Both genders will be injected with two doses of LHRHa to induce ovulation and spermiation. Blood will be collected prior to the priming injection and after ovulation or spermiation as described previously (Dabrowski et al., 2001). Semen and ovules will be collected by stripping after anesthesia (Cardoso et al., 1995). Sperm concentrations will be estimated microscopically using a Double Neubauer Counting Chamber and then spectrophotometrically to develop an appropriate equation (Ciereszko and Dabrowski, 1993). The sperm motility will be evaluated by the same observer and recorded as percent motile sperm. Time of ovulation, fecundity and egg size will be recorded in all females. To test the quality of the eggs, ovules from individual females will be fertilized with pooled semen from three to four males and incubated in separate vertical (conical) incubators of 40 1 capacity. The rate of survival will be assessed at hatching (19 h at 25°C). Hatching success of the embryos will be calculated and expressed as a percentage of the initial number. The plasma concentrations of steroids (testosterone, estradiol-17a, 11-ketotestosterone, and 17,20β-dihydroxy-4-pregnen-3-one) will be measured by radioimmunoassay similar to those used previously (Ottobre et al., 1989) following ethyl-ether extraction. Validation of those steroid assays has already been accomplished in our laboratory for rainbow trout (Dabrowski et al., 1995), paco (Dabrowski et al., 1997), yellow perch (Ciereszko et al., 1997), lake whitefish (Rinchard et al., 2001), and muskellunge (Dabrowski et al., 2000). Assays for those four steroids will be validated in a similarly rigorous fashion for the two target species.

Statistical Analysis: Analyses will be performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). 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 Scheffe's F test (Dagnelie, 1975). 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 $P < 0.05$. Individual tagging of broodfish will allow repeated blood sampling and observation of the evolution of steroid hormones throughout the annual cycle.

Regional Integration

Research efforts being proposed are logical initial steps toward developing sustainable aquaculture in the region. The research will benefit the entire region by providing pertinent information on broodstock reproduction and nutrition. Terra Nuova is the local organization involved in construction and help in local farmer community. Alcantara will be involved in dissemination of both fish produced during this project and information related to production technologies.

Schedule

Objective 1: Growth Performance of Gamitana *C. macropomum* and Paco *P. brachypomus* Larva
July-August 2001, formulation and preparation of the experimental diets
September-October 2001, preparation of the spawning and larval rearing tanks
November-December 2001, induction of spawning of both target species using LHRHa
December 2001-February 2002, feeding experiments for 2-4 weeks duration
February 2002, collection of data on growth and survival
April-December 2002, data analysis and preparation of reports and publications

Objective 2: Changes in Plasma Sex Steroid Hormones in *P. fasciatum* and *P. tigrinum*

July 2001-December 2002, collection of blood samples every 3-6 months
January 2002, preparation of the spawning tanks
February-March 2002, induction of spawning of both target species using LHRHa, collection of blood samples prior to the priming injection and after ovulation or spermiation, evaluation of sperm and egg quality
April-December 2002, analysis of plasma sex steroid hormones by RIA
July-December 2002, data analysis and preparation of reports and publications

Final Report: 30 April 2003

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Survey Study of and Stocking Densities for Tilapia-Shrimp Polycultures

New Aquaculture Systems/New Species Research 3 (10NSR3)/Studies and Experiments/Various

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Introduction

Shrimp ponds have been abandoned in many parts of the world due to diseases, poor management and environmental degradation. Tilapia production, supplemented with low densities of shrimp, in abandoned shrimp ponds may provide an opportunity to develop a sustainable aquaculture system that will support local inhabitants who have not benefited from the shrimp boom in many parts of the world. Polyculture or crop rotation of shrimp and tilapia may even be the modern equivalent of the Chinese polyculture of carp. Tilapia production in former shrimp ponds (with and without shrimp) has increased rapidly in many of the PD/A CRSP locations including Thailand, the Philippines, Honduras, Mexico, Peru and the inland desert of Arizona.

This would be a unique opportunity to take advantage of the strengths of the PD/A CRSPs locations and expertise to conduct cross-cutting research and make a contribution to groups who would be the most likely to understand and benefit from a sustainable production system. Farmers in several locations around the world appear to have demonstrated that tilapia and shrimp can in fact be grown together. There are several technical questions to be answered regarding optimal salinities, stocking densities, feeding rates, disease and parasite infestations, cost-benefits, and environmental impacts. These would be the first replicated trials and technical evaluation of the polyculture. This proposal brings together researchers and industry partners in several countries. The anticipated benefits of the research are: a production system that is more sustainable than the current shrimp farming system, opportunity for local inhabitants to have reliable job opportunities.

Shrimp aquaculture has been devastated in many countries due to a mix of disease outbreaks and decreasing yields. The progression of shrimp aquaculture has followed a familiar pattern throughout the tropics. Initially, farms are constructed in the most appropriate areas. These locations are characterized by good soils with proper pH, appropriate levels of clay, silt and sand, proper elevation, good access to clean water and convenient disposal of waste waters to a location which keeps wastes from being cycled back into the farm. These farms, if managed well, tend to be very profitable. This early success leads others to imitate the process as best they can. This has led to a "gold rush" attitude where excessive numbers of farms are built, often in ecologically fragile areas, especially mangrove forests. From a practical point mangrove forests, in general, are poor sites for shrimp farms. They do not have the proper soils, there is usually poor access to water, inadequate drainage due to low elevation and they are especially susceptible to storm damage.

A related phenomenon is overstocking of an existing farm. After the initial success of a farm, the managers often assume they can increase yields and profits by stocking more shrimp and feeding more heavily. This may work for one or two crops, encouraging even more stocking. But inevitably, the producer overshoots and a disease outbreak occurs because the animals have been overstocked and are stressed under the available environmental conditions in the pond.

In most cases, the farm managers react by increasing water flow through the farm or adding mechanical aeration. These do in fact address the problem but also increase operating expenses and environmental impacts. Added to this situation is the fact that the ponds must be properly maintained and the pond soils managed between crops. Many farms do not properly maintain their infrastructure or their pond environments. When multiple farms in one area reach this stage, there tends to be an environmental overload. The effluent from one farm becomes the supply water for another, the receiving environment cannot process the nutrient rich effluents, leading to eutrophication, and diseases are spread by water transfers, birds, and other vectors. Excessive pumping of water can lead to saltwater intrusion and depletion of freshwater aquifers. Farms that had been wildly profitable with little management, suddenly require more investment and sophisticated management for lower levels of profit. Some farms make the investment to operate in a more sophisticated and sustainable manner, many others just abandon the farm. In many countries the governmental oversight, environmental regulation and protection have been inadequate to avoid this serious ecological damage.

A related problem has been one of land tenure. In many instances investment groups have come in and gained control of coastal lands and hired local inhabitants. These people are usually happy to have the employment and appreciate the infrastructure (roads and electrification) which often accompany the farm. However, when these farms fail, the local inhabitants are often left with no jobs and environmental damages that impair their abilities to return to artisanal fishing or small-scale agriculture. Common environmental damages include salinization of soils, saltwater intrusion, loss of breeding areas for marine species, eutrophication, and changes in the water flow through estuaries.

One technique that has been tested to utilize abandoned shrimp ponds is to convert the pond to tilapia production. There have been several variations of tilapia production including rearing in seawater, brackish water and freshwater. Some have attempted polyculture with shrimp and some are using a crop rotation of tilapia and shrimp (Fitzsimmons, 2001).

Ecological Basis for Tilapia-Shrimp Polyculture

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

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

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

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

From the disease aspect, tilapia seem to provide advantages in several ways. Growers in Ecuador have reported that tilapia will consume dead or moribund shrimp in polycultured ponds. Cannibalism is one of the primary vectors for transmission of shrimp diseases. Tilapia, which do not appear to be susceptible or carriers of these viruses, disrupt cannibalism as a mode of transmission. Tilapia also consume small crustaceans in shrimp ponds. These crustaceans are of concern as potential vectors. Having tilapia directly in the ponds or alternating with shrimp in a crop rotation can be effective for reducing crustacean populations. Bacterial infections also may be impacted by polyculture. *Vibrio* and most other bacterial pathogens common in shrimp culture are gram negative while waters which have been used for fish culture tend to be predominated by gram positive bacteria. Using water from a fish culture pond seems to reduce the prevalence of luminous *Vibrio* bacterial infections in shrimp ponds (Yap, 2001). Growers in Asia and South America have provided anecdotal reports that shrimp production increases due to higher survival in some of these polyculture systems, however, carefully controlled and replicated trials are needed to better study these systems and confirm the results.

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

<u>Methods</u>

The overall project is divided into 5 investigations: 3 survey studies of current tilapia-shrimp polycultures and 2 experiments testing aspects of the farming systems. The intention of the surveys is to examine how farmers in widely separated parts of the world are integrating tilapia-shrimp culture into a sustainable aquaculture system. As often happens, farmers are busy driving technological advances through necessity before the academic community can evaluate and document the most efficient techniques. The unique ability of the PD/A CRSP is our connection to aquaculture developments in Asia, Africa, and the Americas. By allowing aquaculture researchers to work with their domestic farmers and then sharing these findings, we should be able to discern which techniques are the most efficient, from environmental and economic aspects.

The five investigations are:

- 10NSR3A Survey study of tilapia-shrimp polycultures in Vietnam and Thailand
- 10NSR3B Stocking densities for tilapia-shrimp polyculture in Thailand
- 10NSR3C Survey study of tilapia-shrimp polycultures in Mexico and Honduras
- 10NSR3D Stocking densities for tilapia-shrimp polyculture in Mexico
- 10NSR3E Survey study of tilapia-shrimp polycultures in Philippines

The overall project plan is to have several researchers conduct investigations in the form of survey studies at locations in their home countries and possibly a nearby country that is also practicing tilapia-shrimp culture. In several of these countries these investigations would be in conjunction with experiments that would be conducted to determine which conditions, especially stocking densities, are most appropriate in the host country.

Fitzsimmons, Yi, Bolivar, and Contreras, the project PIs, will coordinate the results of the survey studies to develop some international consensus on how these polyculture systems are working. In addition, each host country author will be encouraged to develop and publish his or her national results.

Thailand and Mexico will conduct a two-year experiment, rearing tilapia and shrimp in a polyculture setting. The intent of this experiment is to determine the proper stocking densities of shrimp and tilapia. In some countries the experiments will occur on campus where facilities are available. In other countries where facilities are not available, experiments will be conducted at a cooperating farm.

Details of Investigations A, C, and E

General Survey Methodology: The investigators will develop a contact list of farmers and community leaders in areas that have been impacted by shrimp pond abandonment. Face-to-face meetings will be arranged so that the researchers can visit the sites and document the degree of the problem with the abandoned farms. They will also use this time to contact and visit any farmers who have already tried tilapia-shrimp polyculture. Discussion of techniques, successes, and failures will be key. Finally, the researchers will develop lists of local contacts to share the research results. Especially important will be the social sector contacts who will need to share results with local inhabitants who may wish to utilize ponds which have been abandoned and are available to the local populace.

Survey: The investigator will determine:

a) Which shrimp ponds have been deserted; where, how many, what size, what water source?
b) What were the causes leading to abandonment or low production?
c) Which have been converted to tilapia and tilapia-shrimp culture?
d) Which species or strains of tilapia and shrimp are used?
e) What salinities are used?
f) Who is operating the ponds?
g) What are the inputs and outputs of the system?
h) Are the producers using pond fertilization, pelleted feeds, and aeration?
i) What parasite problems have been encountered and what have been the most successful treatments?
j) Have any diseases been encountered?
k) What are the economic benefits of the polyculture?
l) Will these tilapia and shrimp be marketable as “green” products?
m) What additional follow-up information would the producers like to have?

Investigators will customize the exact informational survey form in concert with one another to insure that the information gathered would be comparable and that local customs and sensibilities are respected.

Density Experiments: The investigators will conduct experiments stocking fish and shrimp at several densities. The researchers will adapt the details of experimental design to be appropriate to the industry and culture techniques in their region.

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Survey of Tilapia-Shrimp Polycultures in Vietnam and Thailand

New Aquaculture Systems/New Species Research 3A (10NSR3A)/Study/Thailand and Vietnam

Objective
Document the existing tilapia-shrimp polyculture in Thailand and the apparent lack in Vietnam, determine the extent of current and potential applications, and determine which techniques are most successful at this point. Producers in Vietnam and Thailand are desperate to supply the EU market, which has required strict environmentally sensitive culture techniques be used. In preliminary research, Yi has not found any tilapia grown in shrimp ponds in Vietnam. An important question will be why have not the Viet farmers adopted the polyculture.

Significance
Thailand was one of the first shrimp producing areas to develop inland shrimp farms utilizing slightly brackish water. These have been some of the first farms to examine tilapia-shrimp polyculture. AIT has existing relationships in Vietnam that will used to make contacts with producers. Vietnam is the most recent country to experience a shrimp farming boom and the bust appears to be in progress. Vietnam has also seen a boom in interest in tilapia production and we expect that the polyculture will be an important technique. Assisting this growth to occur in a sustainable fashion would be a significant contribution.

Quantified Anticipated Benefits
Hundreds of individual shrimp farms with thousands of employees are impacted by diseases and mismanagement. Many of these farms, their owners, employees or cooperative members could benefit if we can document, develop and extend information relating to a more sustainable polyculture system, compared to the existing model of shrimp farming. The number of farms visited will be documented. The findings of the survey will be shared through workshops and through the regular publications and short-courses presented by AIT.

The polyculture survey will be conducted through face to face interviews with farmers in the shrimp producing regions. We will want to document the number of ponds that have been or are being abandoned due to disease and/or mismanagement. We will also determine who is growing tilapia and shrimp in one of the polyculture systems. The target areas will be the coastal areas of Thailand and Vietnam. AIT has an established relationship with production groups and scientists in Vietnam. Fitzsimmons will attempt to join the survey if it can be scheduled in February or March of 2002.

Regional Integration
AIT is a critical resource to aquaculture in Southeast Asia. They publish findings in newsletters and technical journals. AIT also conducts regular short courses in aquaculture as well as traditional training in degree programs. Dissemination of the survey results should bring the information to aquaculture producers across the region.

Schedule
July 1, 2001 to June 30, 2002
Final report will be submitted by June 30, 2002.

Stocking Densities for Tilapia-Shrimp Polyculture in Thailand

New Aquaculture Systems/New Species Research 3B (10NSR3B)/Experiment/Thailand

Objective
The objective of the trials will be to determine optimal conditions for stocking and rearing tilapia and shrimp in a polyculture system. At AIT, relatively extensive techniques will be tested. These would be most applicable to small farmers who would operate family held or small community ponds with minimal inputs. Replicate small ponds will be stocked to model conditions found in the areas impacted by salinization.

Significance
Thailand has been one of the areas hit the hardest by shrimp pond mismanagement. Development of a sustainable tilapia-shrimp system would contribute to environmental restoration and recovery of lost jobs.

Quantified Anticipated Benefits
We expect to have a detailed plan of how farmers could convert operating or abandoned shrimp ponds to a more sustainable production system. This would benefit the farmers who have had to abandon farms along coastal areas as well as inland locations.

Research Methods
Three treatments in triplicate (9 ponds) will be stocked as follows:

a) stock fish at 0/m^2 and shrimp at 30/m^2 each
b) stock fish at 0.25/m^2 and shrimp a 30/m^2 each
c) stock fish at 0.50/m^2 and shrimp at 30/m^2 each

Ponds will be used at the AIT to conduct polyculture trials. Juvenile *Penaeus monodon* will be stocked at a PL40 stage. Nile tilapia (*Oreochromis niloticus*) will be stocked at a one-gram average body weight. The 200-m^2 ponds previously assigned to PD/A CRSP projects will be utilized for these trials. Salinity levels will be adjusted to 5 ppt. Fish and shrimp will be reared in fertilized ponds following PD/A CRSP guidelines and/or fed with commercially available feeds. The trial will extend for 16 weeks when all animals will be weighed. Average growth and survival will be determined for each treatment. ANOVA will be used to determine if treatment means and survival rats are significantly different

Regional Integration
Thailand has developed aquaculture contacts with Vietnam and will be an important conduit of technology and information.

Schedule
July 1, 2001 to April 30, 2003
Final report will be submitted by April 30, 2003.

Survey of Tilapia-Shrimp Polycultures in Mexico and Honduras

New Aquaculture Systems/New Species Research 3C (10NSR3C)/Study/Honduras and Mexico

This survey will be conducted under the direction of Dr. Contreras from the UJAT. Dr. Fitzsimmons will assist with interviews of tilapia-shrimp polyculture in Northwestern Mexico where he has experience and contacts and in Honduras during the aquaculture meetings.

Objective
The objective of this project is to interview shrimp farmers in Mexico and Honduras to document the number of farms being abandoned and why. We would also contact tilapia-shrimp producers in Mexico and Honduras to document details of some basic parameters for stocking and feeding tilapia and shrimp in ponds and raceway systems. Thee results would be published in the scientific literature, at

regional and international aquaculture conferences and shared with others who would be interested to implement tilapia-shrimp polycultures.

Significance
Mexico is a major producer of shrimp with numerous farms on both the Pacific and Gulf of Mexico coasts. The impacts of disease and mismanagement have been severe and alternative production systems that are sustainable are critically important. Honduras is still recovering from Hurricane Mitch and a sustainable aquaculture system is needed to speed recovery.

Quantified Anticipated Benefits
Mexico has been hit by diseases almost as severely as many of the South American producers. The White Spot Virus Syndrome has impacted southern Mexico and Honduras and shrimp producers are looking for alternatives to recover profitability. Many of these farms, their owners, employees or cooperative members could benefit if we can document, develop and extend information relating to a more sustainable polyculture system, compared to the existing model of shrimp farming. Tilapia production is established in Honduras and would benefit from addition of another aspect to the industry. Brackish water culture would add to the total volume of tilapia produced and allow for more efficient use of processing and marketing infrastructure. Our plan would be to document the number of farms visited and the number who later implement changes.

Dr. Contreras will use contacts within the aquaculture communities of Southeastern Mexico and PD/A CRSP colleagues in Honduras. This survey will address one of the widest assortments of tilapia-shrimp polycultures. Mexico has extensive shrimp ponds that have been converted to polyculture as well as intensive, controlled aquaculture systems with sequential raceway culture.

Regional Integration
We hope to use this project to improve contacts between UJAT, Zamorano, and University of Arizona. Zamorano will be hosting the Central American Aquaculture Conference in August of 2001. Tilapia and shrimp will be the primary subjects of the conference and will be the forum to jump-start the project. Dissemination of the survey results should bring the information to aquaculture producers across the region.

Schedule
July 1, 2001 to June 30, 2002
Final report will be submitted by June 30, 2002.

Stocking Densities for Tilapia-Shrimp Polyculture in Mexico

New Aquaculture Systems/New Species Research 3D (10NSR3D)/Experiment/Mexico

Objective
The objective of this project is to develop a tilapia-shrimp production system that will be appropriate to the environmental and economic conditions in Mexico and Honduras. Specifically the research team will determine the some basic parameters for stocking and feeding tilapia and shrimp in ponds and raceway systems.

Significance
Mexico has major shrimp and tilapia production areas. Conversion of shrimp to polyculture operations could increase sustainability from environmental and economic perspectives. It could also provide additional employment and food for local markets.

Quantified Anticipated Benefits
Increased production of tilapia from brackish waters and restoration of shrimp ponds to polyculture ponds would be the primary benefit. We hope to quantify this by recording the number of farms that adopt the technology.

Research Methods
Three treatments in triplicate (9 ponds) will be stocked as follows:
a) stock fish at 0/m^2 and shrimp at 30/m^2 each
b) stock fish at 0.25/m^2 and shrimp a 30/m^2 each
c) stock fish at 0.50/m^2 and shrimp at 30/m^2 each

Fish and shrimp will be fed with commercially available feeds. Shrimp, (*Litopenaeus vannamei*) will be stocked into the tanks at a PL40 stage. Nile tilapia (*Oreochromis niloticus*) will be stocked at a one-gram average body weight. The polycultured animals will be fed at a 10% rate of the total biomass of fish and shrimp per day. The trial will extend for 8 weeks when all animals will be weighed. Average growth and survival will be determined for each treatment. ANOVA will be used to determine if treatment means and survival rats are significantly different

This experiment will be repeated with 40-gram tilapia and 3-gram shrimp to determine how larger fish and shrimp grow in a model of a grow-out system. Feeding rate would be at a 3% rate and the trial will be extended to 12 weeks. Both trials will be conducted at ambient salinity. Additional trials will be conducted with higher salinity levels if time permits. The student will also have the opportunity to gather data from the tilapia-shrimp system at the Genesis farm in Puerto Penasco, Mexico. Fitzsimmons will plan to visit the site in September of 2002.

Regional Integration
Honduras and Panama are regional producers who would benefit from these technologies. Tilapia and shrimp production are growing in the border area of Mexico and the US. Further coordination between US and Mexican scientists and producers is needed. Sharing of graduate students and cooperation on regional conferences are desired goals to improve integration. Through regional aquaculture meetings in Honduras and Mexico and development of e-mail and Web sites, we expect to further integrate aquaculture findings and developments.

Schedule
July 1, 2001 – April 30, 2003
Final report will be submitted by April 30, 2003.

Survey of Tilapia-Shrimp Polycultures in Philippines

New Aquaculture Systems/New Species Research 3E (10NSR3E)/Study/Philippines

Objective
The objective is to determine the extent of shrimp farms being abandoned and if any are now experimenting with tilapia-shrimp polyculture. A session at the 2002 Tilapia Conference to be held in Los Baños in the Philippines would be incorporated.

Significance
The Philippines is one of the major producers of both tilapia and shrimp and likely to be one of the major beneficiaries of the development of sustainable polyculture systems.

Quantified Anticipated Benefits
We plan to identify any existing and potential tilapia-shrimp polyculture sites. Methods and production figures would be published and extended to active and potential practitioners of the polyculture.

A scientist from CLSU will customize the general survey questions listed above to the industry in the Philippines. Face to face interviews will be conducted and the results will be published in technical style, but also developed into extension documents that can be shared with potential practitioners. Fitzsimmons will attempt to join the survey if it can be scheduled in February 2002 in conjunction with the Tilapia farming conference.

Regional Integration

We would integrate the findings from the Philippines with those discerned from Thailand and Vietnam. We hope that by sharing the data and techniques between these three major aquaculture producers, more sustainable systems can be developed. There is also interest in exporting tilapia from the Philippines. Brackish water tilapia would be a likely high value product form that should receive a good price on the international markets. This would further integrate the Philippines into the regional markets for tilapia products.

Schedule

July 1, 2001 – June 30, 2002
Final report will be submitted by June 30, 2002.

Evaluation of Growth and Reproductive Performance of Three Strains of Nile Tilapia *Oreochromis niloticus* Found in Kenya for Use in Aquaculture

New Aquaculture Systems/New Species Research 4 (10NSR4)/Experiment/Kenya

Collaborating Institutions
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Bethuel Omolo

Moi University, Kenya
David M. Liti
Mucai Muchiri

Auburn University
Ronald P. Phelps
Karen Veverica

Objectives

1) Evaluate the production characteristics of three strains of *Oreochromis niloticus*.
2) Determine the reproductive efficiency these three strains.
3) Develop a protocol that will facilitate the evaluation of other *O. niloticus* strains in the future.
4) Train Kenyan biologists in techniques and procedures for species and strain evaluations.

Significance

Tilapia have become one of the most abundantly produced fish in aquaculture. They are produced in over 80 countries. The world production was more than 800,000 MT in 1996 (Lovshin, 1997). Of the tilapias, *Oreochromis niloticus* is the principal species being produced. Its native range includes the Nile drainage from its headwaters in Ethiopia and Kenya to the Nile Delta of Egypt, the Niger drainage, and lakes and stream once historically connected to these drainages.

The cultured stocks of *O. niloticus* are based on a limited number of collections from the wild, often with a limited number of fish in the founding population. The majority of the world production is based on approximately six initial sources: Ivory Coast, Ghana, Egypt-Manzala, Egypt-Ismailia, Sudan-Nile, and Lake George Uganda. Stocks originating from Aswan, Egypt, and Lake Turkana Kenya are being used in a more limited basis. Many of these introductions were made in the late 1960s and early 70s and have been redistributed throughout the world.

As a result of the small founding populations and the prolific nature of tilapia, many of these populations have become highly inbred, limiting the potential for genetic selection. For example, the 50–100 fish of the Ivory Coast strain was introduced into Brazil in 1971. That introduction then became the parent stock for much of the *O. niloticus* cultured in the Americas. In 1988 Teichert-Coddington and Smitherman found a low realized heritability for fast growth in the Ivory Coast strain and attributed that to a small founder stock and subsequent generations of inbreeding. Hulata et al (1986) found no response to selection for rapid growth using the Ghana strain of *O. niloticus*. Al-Hafedh (1994) found that 4th generation Egypt strain *O. niloticus* had promising realized heritability of 0.29 for body weight. Eknath et al. (1993) evaluated four strains recently collected from the wild and four strains with a longer history of domestication and found the three best performing strains were the recently collected ones.

The wide native range of *O. niloticus* permits the opportunity for genetic diversity with in the species. Agnese et al. (1997) analyzed the genetic differentiation of 17 natural populations of *O. niloticus* from both the Niger and Nile systems and found there were three basic clusters, West African, Ethiopian Rift Valley and Nile drainage. Within each cluster there were also distinct subpopulations.

Kenya has a unique opportunity as well as a challenge as tilapia aquaculture becomes more established. The country's geography contributes to three unique drainages where *O. niloticus* is native to one, introduced and well established in another, and present in another where it is not native but beginning to be used in an aquaculture extension program. The Sagana station located in the Tana River drainage where *O. niloticus* is not native and provides the opportunity to evaluate several strains of *O. niloticus* without the worry of contamination of indigenous stocks of *O. niloticus*. The geographical location of the Sagana station offers the potential to evaluate not only those strains which are currently present in the country, but at some point in the future, evaluate other *O. niloticus* strains found in the upper Nile basin. However, the immediate question is which of the strains currently in Kenya is most appropriate for the rapidly growing aquaculture program, based not only on its culture characteristics but bio-diversity considerations as well.

Anticipated Benefits

The proposed work plan will help determine which strain of tilapia should be used where as part of a national aquaculture extension effort. Staff will be trained in techniques for evaluating populations of tilapia that can also be applied to other fish species being considered for aquaculture. The potential is to identify more productive strains of *O. niloticus* that can increase tilapia production worldwide.

Research Design

Kenya

Three strains of tilapia currently present in Kenya will be evaluated to determine if any have unique culture characteristics that favor its use in aquaculture. The stocks to be evaluated will be the Sagana strain, which is being used in the CRSP, sponsored research and extension efforts in the region, the Lake Turkana strain and the Lake Victoria strain. The evaluation will consist of three phases, fingerling evaluation, foodfish evaluation, and reproductive efficiency.

Phase 1: Young fry of each strain will be stocked into small ponds and nursed to 5 g. Growth and survival will be determined for each pond at the end of the production period. The small fingerlings will be stocked into 150 m^2 earthen ponds at 7/m^2 and reared for three months. Ponds will be fertilized with weekly applications of cow manure and inorganic urea. Growth and survival will be determined at the end of that period.

Phase 2: Fingerlings of each strain from Phase 1 will be hand sexed and males graded to obtain a common size for use in a communal culture evaluation. Each strain will be stocked into three replicate ponds/treatment at a density of 2 fish/m^2. All ponds will receive cow manure at 500 kg (dry wt)/ha/wk.

Nine ponds will be managed following a protocol similar to that used in the Kenyan extension program. Cow manure will be added at 500 kg/ha/wk and cassava leaves given daily as a feed at 10% body weight/d. The second set of nine ponds will be managed by adding inorganic fertilizer weekly at 16 kg N and 5 kg P/ha and rice bran given as a feed at 2% body weight/day. All ponds will be sampled every two weeks to determine average weight and seinability of each strain. This phase will be conducted for 5 months after which all ponds will be harvested and growth and survival determined.

In both Phases 1 and 2 the following water quality monitoring program will be conducted: water temperature, dissolved oxygen and pH will be measured weekly in the morning and afternoon at 5, 25, 50, and 75 cm depth. For most analyses, combined water samples encompassing the entire water column will be taken from two walkways extending about 2 m into the pond. Total alkalinity, chlorophyll *a*, Secchi disk visibility, and total ammonia nitrogen will be measured every two weeks.

Phase 3: Brooders will be stocked into 2 m^3 hapas using 8 females and 2 male/hapa and three hapas/strain. Hapas will be monitored every 5 days and females carrying eggs will be collected from spawning hapas and transported to the hatchery where they will be allowed to incubate the eggs. Data will be recorded as to the number of spawns obtained/strain, the number of females that successfully hatch the eggs, and the number of fry produced/gram body weight of female.

Statistical Design: Null hypothesis: There are no differences in the strains in regard to reproductive success, and growth and survival at different points in the life history. Data will be analyzed by ANOVA to determine if there are differences among strains in the above characteristics.

USA

A protocol for strain evaluation will be developed and tested at Auburn University using the two domesticated strains commonly cultured and the potentially genetically most unique Kenyan strain, the Lake Turkana strain. This work will be conducted by a Kenyan as part of a Master of Science degree.

The work will be in two parts, genetic analysis and reproductive performance. A production trial will be conducted as part of a future workplan. A genetic profile will be established following the procedures of Agnese et al. (1997) and Liu et al. (1998).

The reproductive performance will be monitored by stocking brooders of two or more strains into individual 2-m^3 hapas using 8 females and 2 male/hapa and three hapas/strain. Hapas will be monitored every 5 days and females carrying eggs will be collected from spawning hapas and transported to the hatchery where they will be allowed to incubate the eggs. Data will be recorded as to the number of spawns obtained/strain, the number of females that successfully hatch the eggs, and the number of fry produced/gram body weight of female.

Statistical Design: Null hypothesis: There are no genetic differences in the strains or in their reproductive characteristics. Data will be analyzed by ANOVA to determine if there are differences among strains in the above characteristics.

Regional Integration

The proposed work plan will help provide guidelines for evaluation of tilapia strains in the Upper Nile basin. It will help to establish policy regarding fish distribution with in Kenya but also address concerns of other countries in the region. The proposed activities will provide the opportunity for the Kenya CRSP program to provide leadership in the areas of biodiversity and genetic resources.

Schedule

Data collection: 1 July 2001 to 30 September 2002
Data analysis: 1 November 2002 to 30 April 2003
Final report by 30 April 2003

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Reaction of Liming Materials in Pond Bottom Soils

Effluents and Pollution Research 1 (10ER1)/Experiment/Brazil and South Africa

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Jacobus Prinsloo
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Auburn University
Claude E. Boyd
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Objectives

1) Determine the influence of lime application method on total alkalinity and total hardness of pond water.
2) Determine the influence of application method on depth to which agricultural limestone reacts in pond bottom soils of different texture.

Significance

Liming of ponds to neutralize acidity of bottom soils and to increase the total alkalinity and total hardness of pond waters is a well-established practice (Boyd, 1974, 1982; Boyd and Tucker, 1998). Methods for determining lime requirement of pond soils are available and commonly used for determining liming rates (Boyd, 1995). However, there is still no consensus on whether it is more effective to apply liming materials to the bottoms of empty ponds or to wait and apply them over the water surface after ponds are filled. There is also little information on how deep lime reacts in pond sediment over time (Boyd and Cuenco, 1980), and whether the depth of reaction is different when liming materials are applied to the water or to the soil. Also, the influence of soil texture on depth of lime reaction has not been studied, and the possible benefit of tilling pond bottoms on the depth of lime reaction has not been evaluated. The proposed research will provide answers to these questions about pond liming with bottoms of sandy soil or of clayey soil.

The work described here relates directly to research priorities outlined under the Pond Dynamics Research Theme Description. Results of this experiment will provide knowledge on proper application of a commonly used management practice (liming) on exchange of substances between and within soil and water. Improved liming practices are critical to development of effective pond management techniques.

Quantified Anticipated Benefits

When this work is complete, data will be available for coarse textured (South Africa) and fine textured (Brazil) soils regarding the influence of lime application method on neutralization of acidity in ponds. These data will allow us to formulate recommendations on appropriate application methods to maximize effectiveness of lime applied to aquacultural ponds. Beneficiaries of results obtained in this research effort include not only farmers near CRSP sites, but those in neighboring countries as well. Moreover, this experiment will be valuable in solving pond-soil acidity related problems in the US baitfish, channel catfish, and sportfish industries. One PI (C.E. Boyd) has soil projects funded by the Southern Regional Aquaculture Center in the U.S., and this study will complement that effort.

Research Design

Location of Work : Pietersburg, South Africa, and Jaguariuna, Brazil

Pond Facilities: Ponds for use in this work will be ponds on private fish farms in the vicinity of

Pietersburg, South Africa, and Jaguariuna, Brazil. These ponds are 2,000 to 5,000 m^2 in area with average depth of about 1 m.

Culture Period: Brazil: September 2001 through May 2002; South Africa: May 2002 through January 2003

Test Species: Tilapia

Stocking Rates: The stocking rate is not critical to the experiment, and it will be determined by the farmer.

Nutrient Inputs: Feeds and fertilizers will be applied according to the judgment of the pond manager. Liming materials will be applied according to treatments described below.

Water Management: Static ponds with water entering following rainstorms. Water will only be added to replace evaporation and seepage losses.

Treatments: All ponds will be treated with agricultural limestone at a rate sufficient to satisfy the lime requirement of the soil (Boyd 1995). There will be four treatments, each applied to three ponds, each as follows:

- Agricultural limestone applied over pond water surface at beginning of crop.
- Agricultural limestone applied over bottom of pond before pond is filled at beginning of crop, but without tilling.
- Agricultural limestone applied over bottom of pond before pond is filled followed by tilling with disk harrow.
- Control (no lime application).

Sampling and Analyses: Core samples of 20-cm length will be collected from ten locations in each pond with a 5-cm diameter core tube before ponds are limed and at 1-month intervals until harvest. The cores will be cut into 2-cm long segments as described by Munsiri et al. (1995). The soil samples will be oven dried at 60°C in a forced-draft oven. Samples will be pulverized to pass a 40-mesh screen. Soil pH will be measured in 1:1 mixtures of dry soil and distilled water (Thunjai et al., 2001). Exchangeable acidity will be measured by the change in pH caused by adding 5 g soil to 10 ml of buffer solution (Adams and Evans, 1962). Samples taken at the beginning and end of crop will be analyzed for free calcium carbonate (Nelson, 1982). Water samples will be collected from ponds at weekly intervals and analyzed for total alkalinity (acidimetry) and total hardness (EDTA titration) as described by Boyd and Tucker (1992).

Statistical Design and Data Analysis: The null hypotheses to be tested are that the methods of lime application (over water surface or spread over empty bottom) do not differ in their effects on soil pH and exchange acidity and that tilling will not influence the penetration of lime into the bottom soil. The experiment at each location will be arranged as a split-split plot with lime application method (4) as main plots, soil depths (10) as sub-plots, and time (number of sampling dates: 10 for soil pH and exchangeable acidity, 10 for water alkalinity and hardness, and 2 for soil free calcium carbonate) as sub-sub-plots. Each plot/sub-plot/sub-sub-plot combination will be replicated three times. Thus, a total of 12 ponds (four main plots × three replications) will be required to conduct the experiment at each location. Data collected will be subjected to analyses of variance, testing for all main effects and interactions. A probability of greater F ($P > F$) equal to 0.1 will be used to determine significance of treatment effects. Least significant difference will be used to separate treatment means.

The data will be evaluated to determine the depth and rate at which the liming material reacts with the soil by determining in each pond if there are increases in pH, and decreases in exchangeable acidity between adjacent 2-cm layers in each pond. The free calcium carbonate analysis will be used to determine how much limestone remains unreacted at the end of the crop.

Regional Integration

This project integrates well into the regional plans for Africa and South America. The information obtained will be useful for identification of appropriate lime application methods for local conditions in countries surrounding PD/A CRSP sites.

Schedule

September 2001 - Apply treatments and collect initial soil and water samples in Brazil
September 2001 through May 2002 - Collect monthly soil and weekly water samples in Brazil
May 2002 - Apply treatments and collect initial soil and water samples in South Africa
May 2002 through January 2003 - Collect monthly soil and weekly water samples in South Africa
September 2001 through March 2003 - Laboratory analysis of collected samples
April 2003 - Submission of final report

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Elimination of Methyltestosterone (MT) from Intensive Masculinization Systems: Use of Activated Charcoal in Concrete Tanks

Effluents and Pollution Research 2 (10ER2)/Experiment and Study/Mexico

Collaborating Institutions
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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 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 results in the accumulation of MT in sediments and causes the production of intersex organisms and females with altered ovarian development. In systems were substrate was not present, we found higher concentrations of MT in the water and lower (sometimes null) masculinization rates than in those systems with either soil or gravel. We found that charcoal filtration of water from systems where substrate was not present lowers 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.

We propose the use of intensive systems for masculinizing tilapia fry using MT-impregnated food at large scale where the excess of MT is eliminated from the water and the substrate by means of continuous filtration through activated charcoal filters. This method may allow 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. Activated charcoal is cheap and readily available in southern Mexico, and it is well known by aquaculturists that it can be reactivated by sunlight exposure. Sunlight treatment of the activated charcoal may bring another benefit, i.e. the breakdown of MT due to steroid photosensitivity.

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 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

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

The use of activated charcoal filters in intensive systems as an alternative treatment for masculinizing tilapia will improve safety in handling masculinizing steroids, produce clean effluents, and potentially increase efficiency of exposure.

Research Design

Experiment A: Elimination of MT from the Water of Intensive Sex-Inversion Systems

Site: 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^2), 3 grow-out ponds (200 m^2), a total of 200 females and 65 males for production of fry.

Culture Period: 3 months or until tilapia reach sampling size (3 cycles)

Stocking Density: 3,000-4,000 fry/m^2

Test Species: Nile tilapia (*Oreochromis niloticus*)

Nutrient Inputs: None

Water Management: Water will be filtered through a 200-l tank containing an activated charcoal filter bed at a rate of 50 l/min. Water will be recirculated through the rearing tanks in all systems. The MT tanks will receive MT-treatment recirculated water; the control tanks will receive control recirculated water. A 25% water exchange will be performed twice a week.

Sampling schedule: The experiment consists of four treatments:

- fry fed MT at 60 mg/kg food for 28 days; water filtered through a 0 kg activated charcoal bed
- fry fed MT at 60 mg/kg food for 28 days; water filtered through a 5 kg activated charcoal bed
- fry fed MT at 60 mg/kg food for 28 days; water filtered through a 10 kg activated charcoal bed
- fry fed control food for 28 days in grow-out pond.

Because of tank availability, each treatment will consist of a single experimental unit. The entire experiment will be repeated three times and used in the data analysis as pseudoreplicates. Water

(12 ml) samples will be collected from the sex-inversion tank on days 0, 7, 14, 21, 28, and 35. Five sampling locations will be randomly selected each time. Samples will be frozen (–20°C) and preserved until processing. Activated charcoal used in the treatment will be exposed to direct sunlight for 24 and 48 hours, and samples will be collected at different times for MT detection. All samples will be extracted using ether and the concentration of MT determined by radioimmuno-assay (RIA; at OSU). At the end of 3-month grow-out period, a subsample of the tilapia in each experimental unit (100) will be killed with an overdose of anesthetic (MS-222) to determine if the treatment with MT resulted in masculinization. The following water quality parameters will be measured daily: pH, DO, and temperature. Activated charcoal used in the treatment will be exposed to direct sunlight for 24 and 48 hours, and samples will be collected for MT detection.

Statistical Methods and Hypothesis: $H_0 1$: MT is detectable in water at any time during and 1 week after treatment of tilapia fry with MT-impregnated food independently of the amount of activated charcoal used in the filter. This part of the study is descriptive and therefore, statistical analysis is unnecessary for testing the null hypothesis (i.e. failure to detect any amount of MT in water will be sufficient for rejecting the null hypothesis). $H_0 2$: Administration of MT feed to tilapia held in systems with different amounts of activated charcoal results in treatments with the same sex ratios. The efficacy of MT treatment will be tested comparing between MT-fed and control treatments by Chi-squared test.

Schedule: Data collection, 7/01-6/02; Technical report, 6/30/02

Study B: Implementation of Safe Intensive Sex-Inversion System in Local Tilapia Farms
Site: Filtering systems will be evaluated at the following farms:
a) Ejido Rio Playa, Comalcalco, Tabasco, México
b) Piscifactoría Teapa, Tabasco, México
c) Centro de Producción y Fomento Piscicola "Lacandona," Chiapas, México

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

Laboratory and Pond Facility: All farms have concrete tanks, grow-out ponds (200 m^3), and broodstock.

Culture Period: 3 months or until tilapia reach sampling size (3 cycles)

Stocking Density: 3,000-4,000 fry/m^2

Test Species: Nile tilapia (*Oreochromis niloticus*)

Nutrient Inputs: None

Water Management: Water will be filtered through a 200 l tank containing an activated charcoal filter bed at a rate of 50 l/min. Each tank will have its own filter. Water will be recirculated through the rearing tanks in all systems. 25% water exchange will be performed twice a week.

Sampling Schedule: Based on the results from Experiment A, the treatment that results in the maximum percentage of males and the minimum amount of MT in the water will be selected to be used at the farms. Experimental treatments will be as follows:

- fry fed MT at 60 mg/kg food for 28 days; water filtered (best treatment from experiment A)
- fry fed control food for 28 days in grow-out pond.

If possible, replicates will be used. Water (12 ml) samples will be collected from the sex-inversion tank on days 0, 7, 14, 21, 28, and 35. Five sampling locations will be randomly selected each time. Samples will be frozen (–20°C) and preserved until processing. All samples will be extracted using ether and the concentration of MT determined by RIA (at OSU). At the end of 3-month grow-out period, a subsample of the tilapia in each experimental unit (100) will be killed with an overdose of anesthetic (MS-222) to determine if the treatment with MT resulted in masculinization. The following water quality parameters will be measured weekly: pH, DO, and temperature.

Statistical Methods and Hypothesis: $H_0$1: MT is detectable in water at any time during and 1 week after treatment of tilapia fry with MT-impregnated food independently of the use of activated charcoal filters. This part of the study is descriptive and therefore, statistical analysis is unnecessary for testing the null hypothesis (i.e., detection of any amount of MT in water or substrate will be sufficient for rejecting the null hypothesis). $H_0$2: Administration of MT-feed to tilapia held in systems with activated charcoal results in treatments with the same sex ratios as the controls. The efficacy of MT treatment will be tested comparing between MT fed and control treatments by Chi-squared test.

Schedule: Data collection, 7/01-6/02; Technical report, 6/30/02

Regional Integration

If the results of the Study B are successful, we will exchange information with CRSP Honduras Project researcher Daniel Meyer at Escuela Agrícola Panamericana (Zamorano). We have already discussed with the Peru and Thailand PD/A CRSP research teams our intentions to exchange information among project scientists and for potential technology transfer.

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Environmental Impacts of Cage Culture for Catfish in Chau Doc, Vietnam

Effluents and Pollution Research 3 (10ER3)/Study/Thailand and Vietnam

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Objectives

1) Investigate the cage culture system and its related environmental conditions.
2) Determine the quality and quantity of pollutants produced by cages.
3) Detect the fate of pollutants in the river.
4) Recommend methods for pollution mitigation in cage culture.

Significance

Cage culture is commonly practiced worldwide in both freshwater and marine environments, including open ocean, estuaries, lakes, reservoirs, ponds and rivers (Beveridge, 1987). In Southeast Asia, cage culture plays an increasingly important role for fish production, which involves many small-scale farmers in Vietnam, Cambodia, Indonesia and Thailand (Liao and Lin, 2000). However, the environmental impact of cage culture is often ignored and rarely subjected to research or investigation.

The cage culture of *Pangasius* catfish originated many years ago in Cambodia and has spread widely to other Indochinese countries. The best known areas for intensive catfish production from cage culture are in An Giang and Dong Thap provinces (Andriesz, 2000), where annual production in the year 2000 was 42,000 and 20,000 tonnes by 3,000 and 2,000 cages, respectively (Anon., 2000). The total fish production has increased 5 fold between 1995 and 2000. Most catfish cages are concentrated along the banks of Mekong River near Chau Doc, which is also the major supplier of fingerlings for cage stocking. The main species being cultured are *Pangasius bocourtii* and *P. hypothalamus* along with minor species like *Chana micropelpte* and *Puntius gonionotus*.

Cage cultured fish are entirely dependent on formulated diet (Phuong, 1998), and the waste produced from this consumption is released directly to the river. This results in cage culture contributing nutrients, organic matter (BOD), and turbidity that causes deterioration of water quality and biota downstream (Pillay, 1992). The quantity of wastes discharged from a fish cage depends on the quantity and quality of feed inputs (Cowey and Cho, 1991). With relatively low protein and high carbohydrate diet, nutrient loading from culture of *Pangasius* waste is likely to be much lower than for salmonid culture in cages. However, waste in the form of organic matter, particulate matter and suspended solids may result in major heavy sediment accumulation and BOD near the site of cage culture systems. As a result of rapid expansion in cage culture in Chau Doc, water quality is reported to have deteriorated so much that fish disease outbreaks have occurred when the river is low and water flow sluggish during the dry season. While research has been done on seed propagation and diet formulation, little effort has been expended on mitigation of environmental impacts and improvement of water quality to ensure sustainability of cage culture in Chau Doc.

Anticipated Benefits

The results of this research will provide information directly to farmers for better management their cages based on water quality and hydrological features. It will also enable managers to estimate carrying capacity of the river for cage culture, which is essential to allow governmental agencies to establish policy and plans for cage culture development. The information will provide some evidence on the degree of water quality deterioration. It will benefit thousand catfish cage farmers in Chau Doc in long term.

Research Design

Location of the Work

Laboratory and logistic support: Can Tho University
Field site: Chau Doc, Mekong River, Vietnam

Study Plan and Methodology: Social and economic aspects of cage farmers will be investigated with among 20% of farmers using a set of designed questionnaires. A structured checklist and open-ended type of questionnaires will be designed. The questionnaires will consist of socioeconomic characteristics of farmers, cage culture practices, investment cost and return, problems and other information.

Seasonal pattern of hydrological features will be measured monthly in the cage culture area, including flow rate and depth fluctuation in open river water and inside the cages.

Water samples will be taken monthly in 12 locations in the cage culture area along the river and in 12 cages for analyzing ammonia, suspended solids, organic matter, total nitrogen and total phosphorus following standard CRSP methods. DO, pH, and temperature will be measured during the sampling time. Cages will be selected to represent the variety of systems in the region.

The organic matter and nutrient deposition pattern in the river bed near cage culture operations will be determined by taking sediment samples at 12 locations along the river near the 12 cages sampled (Boyd, 1995).

Feed will be analyzed for its proximate composition and N and P nutrient contents as well as feed inputs. Mass balance equations of inputs and accumulations in animals will be made, and the resulting loss of materials to the water column estimated.

Relevant environmental regulations will be reviewed and governmental agencies interviewed for the information about regulations, enforcement and farmers' attitudes.

Regional Integration

As cage culture is widely practiced in the Southeast Asian countries, this case study will provide an important methodology as protocols of environmental impact.

Schedule

August 2001 to September 2002
Report submission: January 2003

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Regionalizing Training and Technical Assistance for Nongovernmental Organizations

Appropriate Technology Research 1 (10ATR1)/Activity/El Salvador, Honduras, and Nicaragua

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Objectives

Provide technical training and outreach activities to build nongovernmental (NGO) institutional network for aquacultural development in Latin American countries. Specific objectives of the investigation are:

1) Train NGO extension agents in the fundamentals of tilapia culture utilizing low-cost inputs as a tool to be used in rural development activities in Nicaragua and El Salvador,
2) Train NGO extension agents in the use of Web-based information delivery system that provide information and decision making process for advising small- and medium-scale farmers, and
3) Prepare and distribute useful information on tilapia culture using low cost inputs for implementing and providing technical assistance to rural farmers in Nicaragua and El Salvador.

Significance

The potential for aquaculture in Honduras, and in the entire Central American region is quite high. For example, a previous regional-scale study (Kapetsky and Nath, 1997) suggests that from 45-90% of the land area in Central America is very suitable for commercial farming, and that 35-90% of the land area is very suitable for small-scale aquaculture. One of the serious limitations impeding attainment of this potential is the limited institutional support for aquaculture development (e.g., extension services, training/research opportunities, marketing channels, etc.) in Honduras (Veverica and Molnar, 1997). The lack of institutional support for aquaculture is also evident from findings that roughly a third of aquaculture farmers in Honduras cite lack of understanding of fish farming as a major obstacle to increased yields (Molnar et al., 1996). These issues are not unique to the country, but exist in many countries across Latin America (Martinez and Pedini, 1998).

The overall situation vis-à-vis governmental support for aquaculture in Honduras suggests that now is an opportune time to strengthen institutional capability in the nongovernmental sector to ensure that the infrastructure for small and medium scale aquaculture producers will be in place for the foreseeable future. Consequently, the purpose of this project is to provide training and outreach activities that will institutionalize aquaculture development in Honduras and Latin American countries with the nongovernmental institution (Zamorano, the Escuela Agrícola Panamericana in Honduras) playing a central role, in collaboration with the University of Georgia and Auburn University.

Fish culture can provide inexpensive animal protein and extra income for rural farmers in Central America. There are proven techniques and tested recommendations for utilizing low-cost inputs for growing tilapia in tropical and subtropical areas of the world (Meyer and Mejía, 1993; Meyer el al., 1997). Much of these techniques and recommendations have been developed by CRSP research efforts (Green et al., 1994). Currently, fish culture is not part of the traditional production systems in Latin America. An effective extension program in fish culture requires a long-term commitment to provide useful information for decision-making and continued motivation of beginning fish farmers.

This project builds on the experience gained in the current Pond Dynamics/Aquaculture CRSP Honduras project and the prior 14 years of research experience, during which time CRSP researchers have built a pool of trained individuals, developed contacts with several NGOs, undertaken the development of Web-based information delivery system, and build capacity in Zamorano and RDS-HN to the extent that they will play a significant role in regionalizing training of NGOs thereby build capacity in Latin American countries. Therefore, we propose to partner with Zamorano and RDS-HN to provide technical assistance and to engage in training and outreach activities that will build the non-governmental institutional network for aquacultural development in Latin American countries and support governmental initiatives in aquaculture. Zamorano is well positioned to provide the leadership, collaboration, and institutional integrity necessary and RDS-HN is poised to provide information through tools of information technology to sustain development through training and technical assistance to small- and medium-scale farmers long after the PD/A CRSP has completed its work. Zamorano is at the heart of our vision of the PD/A CRSP exit strategy.

Due to the vast number of small- and medium-scale farms and time and resource constraints of the CRSP, it is not feasible to interact directly with farmers to offer technical assistance for culturing tilapia. The chosen strategy in the Honduras project has been build capacity in Zamorano, strengthen NGO capabilities and "to train the trainers" to offer assistance in this field on a long-term basis. We propose to continue this strategy which proving successful as is evident with the effectiveness RDS-HN in enabling distribution of information to several hundred NGOs.

There are a multitude of NGOs operating in the Latin American countries. Many of these organizations include fish culture in their rural development efforts but have limited access to pertinent information for promotion and implementation of effective extension efforts. Most NGO extension agents have limited knowledge and experience of appropriate fish culture techniques. The significant effort in Honduras to strengthen the capabilities of numerous NGOs in tilapia culture can now be extended to Nicaragua and El Salvador that have similar topographical and climatic conditions as Honduras. Both countries are poorly developed and their populations suffer from widespread malnutrition. We expect other countries in the region to follows from this work.

Quantified Anticipated Benefits

The overall benefit from this investigation will be continued capacity building of Zamorano and NGOs there by enabling these institutions to provide for continued development of small- and medium-scale aquaculture in the Latin American region.

The proposed work will increase knowledge and capabilities of several dozen NGOs and extension agents in tilapia culture. By receiving hands-on training with WIDeST, they will also learn the use of Web-based system for retrieving information, virtual meetings format for receiving inputs to identify needs, and methods by which to make decisions. Understanding factors affecting economic development and learning the value of assessing availability of natural resources will be valuable in providing advise to small- and medium-scale farmers.

Activity Plan

Activities planned in this investigation are to provide training and technical assistance in the selected Latin American countries for culturing tilapia. These activities includes providing information from PD/A CRSP results on tilapia production and management, local markets, water supply analysis and pond design, and the use of the Web-based information delivery system for systematic decision-making. Specific activities include:

1) Initial exploratory meetings with select farmers, NGOs and policy makers in Nicaragua and El Salvador to survey range of NGOs operating in the region, identify appropriate groups for training, and topics of training,

2) Conduct one- to two-day training in Nicaragua and El Salvador giving overview of tilapia culture utilizing low-cost inputs as a tool to demonstrate the potential in small- and medium-scale farm settings,
3) Prepare technical materials and information sheets on tilapia for the target groups identified in the region,
4) Include in WIDeST aspects relevant to tilapia and demographic and natural resource information useful in the region and use WIDeST for training and workshops described in 2 and 5,
5) Provide five-day long training workshop in Nicaragua and El Salvador with topics on tilapia culture, nutritional aspects, economics and local markets, water supply and natural resources, Web-based information systems and methods of systematic decision making.
6) Capstone presentation in a workshop setting in Honduras on the topics in above 5 as well as full hands-on experience in use of models for tilapia culture, pond design and WIDeST.

Regional Integration

The proposed work in this activity is primarily focused on developing capacity of Zamorano and RDS-HN to provide regional leadership in training and technical assistance in Latin American countries. The training workshops and meetings are targeted to extend CRSP experiences in Honduras to Latin America. The Web-based technology and WIDeST show great promise for achieving this goal. The training sessions and hands-on experiences of farmers, NGOs and policy makers with WIDeST will go a long way in using relevant information for decision making as well as regionalizing of tilapia culture in Latin America and beyond.

Schedule

We plan to hold several training sessions as follows:

- Dan Meyer from Zamorano make initial contacts in Nicaragua and El Salvador and he conduct exploratory meetings with farmers, NGOs, extension agents, and policy makers. [October – November 2001]
- Dan Meyer, Bill Tollner and Brahm Verma present a one-day session to present an overview of tilapia culture, water supply analysis and pond design and use of the Web-based system. Also, receive inputs from stakeholders which is incorporated in research in the two companion investigations. [January – March 2002]
- Prepare literature and update WIDeST for use in the region and to be presented at training sessions in Nicaragua and El Salvador. [March – September 2002]
- Dan Meyer, Bill Tollner, Brahm Verma and Joe Molnar, and RDS-HN staff conduct one-week long workshops, one each in Nicaragua and El Salvador with hands-on experience with tilapia culture, water supply and pond design models, and the Web-based information system. [October – December 2002]
- Conduct a capstone training workshop in Tegucigalpa giving extensive exposure to the work accomplished with PD/A CRSP. This exiting event will complete work on expectations that the increased capacity of Zamorano and RDS-HN and the developed Web-based system have created the enabling environment for incorporating of fish culture as an alternative for economic development in Latin American countries. [January-March 2003]

Submit final report. [April 2003]

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Institutionalizing Web-based Information System for Tilapia Culture in Latin America

Appropriate Technology Research 2 (10ATR2)/Study/El Salvador, Honduras, Nicaragua, and Panama

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Objectives

To complete the undertaking of Web-based Information Delivery System for Tilapia (WIDeST) which makes available to stakeholders (farmers, NGOs, policy makers and other change agents), the knowledge of tilapia culture using low-cost inputs, relevant demographic and natural resource data, facilities for real-time on-line conversation for exchanging information, and tools for employing decision making process.

Specific objectives of the proposal are to:

1) Collect all aspects of information needed for evaluating tilapia culture as an alternative for small- and medium-scale farmers;
2) Organize and present information in WIDeST that is usable by extension agents and NGOs to train small-scale farmers;
3) Develop methods for receiving stakeholder input, conducting live conversations through the Web among farmers and NGOs to share experiences and identifying needs; and
4) Incorporate decision-making methods in WIDeST useful for employing systematic process for developing and evaluating decision alternatives.

Significance

The concept of an enabling environment has been identified as a key prerequisite for sustainable aquacultural development (Shehadeh and Pedini, 1997). Experiences in natural resource management initiatives for the hillside regions of Latin America (CIAT, 1997) suggest that creating partnerships among stakeholders involved in managing and/or using natural resources is part of the process of fostering an enabling environment; the other aspect is to adopt an integrated decision-making framework for use in such environments (Nath et al., 1999). The latter framework (referred to as concurrent decision making) has been applied in workshop settings for natural resource management initiatives in Central America (Honduras, Nicaragua).

We realized the need for a systematic method for enabling communication to reawaken the dialogue. In this context the University of Georgia and its collaborators from Zamorano and Auburn University identified the work of and potential capabilities of Red de Desarrollo Sostenible in Honduras (RDS-HN). The Red de Desarrollo Sostenible-Honduras (RDS-HN) was created with the initial grant from the United Nations Development Programme (UNDP) in response to the 1992 Earth Summit which mandated assistance to "developing" countries for establishing in-country Sustainable Development Networks (SDNs). These networks were envisioned to provide infra-structural support for rapid communication through electronic information technology. The RDS-HN was among the first to establish network and now provides the Internet services to over 700 customers. Similar SDNs have been created in other Latin American countries, e.g., Costa Rica, Dominican Republic, Nicaragua, Panama, Guatemala, Haiti, Mexico, Guyana, Bolivia and Columbia. Together these SDNs can constitute a formidable information network to facilitate exchange and contact among farmers, government and non-government organizations as well as private entrepreneurs. Thus, we believe there is a unique opportunity to pursue capacity building and institutional strengthening for aquaculture in Latin America through the use of SDNs and a Web-based information delivery system. The task in this phase of the PD/A CRSP is to identify and implement those steps necessary to ensure that small- and medium scale fish farms can be sustained as a productive enterprise in Honduras. Implementing small and medium scale aquaculture on a widespread and sustained basis is a long-term process (Harrison 1991, Molnar et al., 1991). Consumer demand and dietary preferences are not obstacles to fish culture in Honduras nor are sales problems necessarily a reason for abandoning ponds. Tilapia is widely acceptable as a consumer item. In a 1996 survey, a majority of Honduran farmers noted "my understanding" as the major obstacle to obtaining larger harvests from their ponds (Molnar et al., 1996). Thus, it is vital that this project fortifies partnership between Zamorano and RDS-HN and their ability to sustain aquaculture development in Honduras. This work will contribute to diminished dependence of small-scale farmers on technical assistance from outside sources as it will enable host country NGOs and private firms to provide services and technical assistance locally.[1]

Quantified Anticipated Benefits

The unprecedented accessibility of knowledge for tilapia culture, data on availability and constraints of natural resources and systematic method of decision-making by farmers, NGOs, extension agents, government policy makers, financial institutions and investors will create an enabling environment for small- and medium-scale farmers in Latin American countries. Stakeholders will be able to interact in a participatory manner that would provide sound basis for management decisions on farms and for developing policies and plans for aquaculture development in the region. The number of users will be a clear indicator of the benefit.

Another benefit will be the use of WIDeST for providing training and workshops to farmers, NGOs and representatives other change agencies. Use of WIDeST for training students and technicians in education institutions such as Zamorano and staff of NGOs such as RDS-HN in tilapia productions and systematic decision making methods will enable capacity building in host countries.

Regular inputs of stakeholder experiences and virtual meeting through "live chats" facilities will empower host country farmers and NGOs and enable them in influencing policies and decisions in their favor.

Input from researchers and identification of new research needs will be an important benefit. It is likely that knowledge and experiences from other areas will benefit decisions in Latin American countries through WIDeST.

The above and other related benefits are quantifiable by proper recording of number of individuals, organizations/agencies and training sessions using WIDeST and the number of decisions influenced by information in WIDeST.

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[1] Honduran rural women play an important role in agriculture, especially in the peasant and small farmer sectors, working an average of four hours a day in crop and livestock activities. Women are responsible for establishing a survival strategy for the household unit. Women, who bear the entire responsibility for agricultural production, head about 20% of rural households. Development policies, however, still consider men as producers and women as responsible only for household tasks. Women are primarily responsible for vegetable gardening and small livestock - poultry, pigs and goats. While little data is available on the gender division of labor in agriculture, women take part in most activities and predominate in food processing. In fisheries, women also work primarily in processing activities. Women are responsible for household tasks, including water supply. Men contribute significantly to fuel wood collection. In households in which women are active in agricultural production, men and women often share the decision-making. Men predominate in decisions in regard to type of crops, varieties, and fertilizers, while women generally have a greater say in family expenses and pricing of produce (Fleck, 1996). <www.fao.org/sd/wpdirect/WPan0005.htm>

Research Design

The work will be conducted simultaneously and collaboratively at the University of Georgia, Zamorano and by employing the services and facilities of RDS-HN in Honduras. Auburn University will be directly involved in providing expertise for information in fish culture. Much of the research plan will be receiving and organizing information and data to include the following important features in the Web-base system.

1) Easy access to the desired information, i.e., the packaging of information is intuitive and the access to the Website is straightforward from other relevant Internet sites where a stakeholder might normally look for information.
2) Ready and rapid availability of information in term of time, i.e., from the time a question is presented to the time a satisfactory answer is made available. This capability should be available 24 hours a day.
3) Few barriers should exist for receiving information and for posting questions, i.e., low cost, not requiring advanced/sophisticated hardware and software, no regulation barriers.
4) Confidence in the accuracy of data, information and knowledge is essential. Accuracy and updates with the latest data on weather, natural resources, production practices, markets, demographics, regulations, etc. and knowledge for guiding in the selection of pond site and design are necessary requirements.
5) Mechanisms that identify a facilitator(s) as a liaison for those who are not able to directly use WIDeST so that the facilitator(s) becomes the conduit of delivery. For example, NGO and extension agents can receive information from WIDeST and provide advise to small-scale farmers.
6) Mechanism for users to:
    - ask questions specific to their needs and receive response in a reasonable time from other users as well as experts;
    - suggest improvements for information delivery system; and
    - create a network of stakeholders for planned discussions with a facilitator in a virtual conference environment.

The following important features for easy and effective maintenance and improvement are critical to long-term use of WIDeST:

1) Easy updates of data, information and knowledge in terms of time and cost.
2) Mechanisms that make identification of available new information easy as well as receiving information in format compatible for rapid updates.
3) Capability to respond to new and unexpected needs on a timely basis.
4) Capability to post reminders for reoccurring events and activities.
5) Ability to assess performance of components of WIDeST for meeting stakeholder needs, impact of tilapia on economic development and needs for improvements.
6) Ability to identify users and potential new sources of support from stakeholders for continued maintenance expenditures.

The following connectivity capabilities are important features:

1) User identification is obtained and contact information stored for future use.
2) User readily able to send E-mail to “webmaster” and “experts” with questions and comments.
3) User is easily able to connect to other relevant websites giving pertinent information on fish production, weather forecast and economic forecast.
4) User is easily connected to sources on related topics, such as, the role of fish in human diet, food security and nutrition issues.
5) User are connected to relevant market forecast.
6) Users are connected to important announcements and upcoming events.
7) Users can connect with others in a virtual conference and training sessions.

The overall method will be to continue identification of needs from the farmers and other stakeholders, receive expert knowledge and best practice recommendations and organize all information for systematic decision-making for tilapia culture. Finally, using focus groups for both identifying needs and then for evaluating the effectiveness of WIDeST will be an important method.

Regional Integration

The proposed work will achieve regional integration primarily through the Sustainable Development Networks (SNDs) in Latin American countries. Additionally, the training sessions and workshops planned in the region for extending this work are described in the complementary investigation (activity) described in this proposal. Lastly, focus groups and virtual conferences using "live chat" facilities of WIDeST will extend this work's effectiveness in the region of Latin America.

Schedule

Web-based information system, WIDeST has URL: <www.acuacultura-ca.org.hn> and it currently has limited information on tilapia culture. The schedule of work in this project will include the following:

- A more thorough collection of data and knowledge relevant for tilapia culture in Latin American countries. [September 2001 to December 2002]
- A thorough collection and linking of demographic, economic, marketing, climatic and natural resources information. [September 2001 to December 2002]
- Conduct one focus group each in Honduras, Nicaragua, El Salvador and Panama to receive input from the stakeholders and evaluate effectiveness of the proposed Web-based system. [January 2002 to March 2002]
- Update WIDeST based on focus group input by modifying both content and accessibility (search steps for) relevant information. [April 2002 to September 2002]
- Conduct two virtual meeting through live chat facilities with stakeholders from several Latin American countries. [April 2002 and August 2002]
- Final presentation of WIDeST and evaluation of effectiveness in a daylong session with stakeholders and policy makers. [January to February 2003]
- Final report and publications. [April 2003]

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Diversification of Aquacultural Practices by Incorporation of Native Species and Implementation of Alternative Sex Inversion Techniques

Appropriate Technology Research 3 (10ATR3)/Study and Experiments/Mexico

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Objectives

1) Establish broodstock lots of tropical gar (*Atractosteus tropicus*) and mojarra castarrica (*Cichlasoma urophthalmus*).
2) Produce 100% male castarrica fry.

Significance

In the tropics, two approaches for utilizing the large diversity of fish species offer potential for aquaculture development: 1) species with moderate growth rates and excellent markets and 2) species with sexual dimorphism and large demand for the "attractively colored gender" in aquarium stores. 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 is the Nile tilapia and little effort has been devoted to develop options with local species. Worldwide, 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. The protocols for the masculinization of 47 species and the feminization of 31 species have been developed so far (Pandian and Sheela, 1995). Among the steroids used, 17α-methyltestosterone (MT), trenbolone acetate (TA) and estradiol (E2) have proven effective for masculinizing (MT and TA) and feminizing (E2) fish fry.

At the Laboratory of Aquaculture in Universidad Juárez Autónoma de Tabasco (UJAT), the production of tropical gar fry has been successful over the last five years (Márquez-Couturier, 2000). A previous study demonstrated that females reach larger sizes than males (Contreras-Sánchez and Alemán-Ramos, 1987), but little is known regarding the advantages of producing 100% female populations. The tropical gar is considered a delicacy dish in Southern Mexico and an integral part of local culture—analogous to the importance of the salmon in the Northwest of the US. Gar is sold in every seafood restaurant in Tabasco and the pressure on its populations has lead to the listing of the species as "susceptible" (Contreras-Sánchez, 1990).

The mojarra castarrica has been considered as one of the few native species that may perform as well as tilapias in aquacultural systems in terms of growth rate and achievable harvest size (Martinez-Palacios and Ross, 1994). As in tilapias, males grow faster and larger than females; however, little has been done about the production of 100% male populations and the few studies that have been carried out resulted in less than 70% male populations (Hernández-Betancourt, 1988).

The growing international demand and high prices for tropical species in aquarium stores has already created great pressure on the natural populations. As an example, in Japan, tropical gar juveniles have a wholesale price of US$15 per fish. Many of the specimens sold in aquaria are wild-captures and very few are produced in aquacultural facilities. In the last two years, the laboratory of aquaculture has started research on native species that show potential for being incorporated into intensive culture systems. We are currently studying the early life history of tropical gar, mojarra castarrica, mojarra paleta, and mojarra azuleja, and we are working on a manual for the culture of these species. These on-going studies involve the time required for embryonic and larval stages, time of sex differentiation, feeding habits, etc.

Possible alternatives to the problems encountered for sex inversion of new species of fish in aquaculture are the administration of steroids via bioencapsulation and immersions. The first technique offers the advantage that the larvae of the two species proposed for this study strongly prefer live food over artificial diets. This technique has been used for administering chemotherapeutics and nutrients to fish, shrimps and prawns (Roque et al., 1998; Touraki et al., 1999) and is considered as a good alternative method to incorporation of hormone into manufactured feeds. The second alternative offers the possibility of feminizing or masculinizing the proposed species without the need of feed containing hormones. Because masculinization of fry by oral administration of MT is considered the most successful method employed to masculinize fish fry (Green et al., 1997), we intend to compare this protocol with the administration of bioencapsulated trenbolone acetate (TA) and immersions in TA. We will also validate the bioencapsulation technique using tilapia as a model species. For feminization of tropical gar, immersions in estradiol (E2) will be evaluated. We will use the same protocol that we have used to masculinize Nile tilapia (i.e., 3–5 h immersions at the onset of feeding; Contreras, 2001) after determining the time at which the gonads enter the labile period (work which is currently underway and expected to yield results within 6 months).

Extension workshops and outreach material will be developed to increase the awareness of both producers and the public about the economic potential and the environmental benefits associated with the diversification of aquaculture by incorporating native species of fish.

Anticipated Benefits

Incorporation of native species to aquaculture is needed. The development of technological packages for native species will benefit fish farmers in Southern Mexico and Central America. Particularly, this study will focus on the development of techniques that allow sex inversion in those species of economic importance. These techniques are of considerable importance in promoting diversification of aquaculture species. Furthermore, the assessment of techniques that do not require the use of steroid-treated food may provide aquaculturists with a safe and cost effective alternative, because immersion will require substantially shorter exposure periods and the steroid will be contained for controlled filtration or biodegradation. 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.

Research Design

Study A: Enrichment and Detection of E2 and TA in Artemia Nauplii

Site: Experiments will be conducted at UJAT, and samples processed at Oregon State University, Corvallis, OR.

Methods: Immersion of Artemia nauplii in steroids (E2 or TA) 1,000 µg/l. Two methods for Artemia enrichment will be evaluated (bioencapsulation via oil diets and immersion in steroid solutions).

Laboratory and Pond Facility: UJAT; 20 4-l glass jars. Oregon State University; Laboratory and equipment needed for RIA and HPLC.

Culture Period: 12 hrs

Stocking Density: 1 million nauplii/L

Test Species: Artemia salina (and/or *Daphnia magna* depending upon Artemia availability)

Nutrient Inputs: None

Water Management: None

Sampling schedule: The experiment consists of three treatments:
- Artemia nauplii immersed in 1,000 µg/l E2
- Artemia nauplii immersed in 1,000 µg/l TA
- Artemia nauplii immersed in Ethanol vehicle

Each treatment will consist of three replicates. Water with Artemia nauplii (50 ml) samples will be collected from the glass jars at 0, 2, 4, 6, and 12 hours. Nauplii will be washed in nanopure water, dried and samples will be frozen (–20°C) and preserved until processing. All samples will be extracted using ether and the concentration of steroid determined by RIA (E2) or HPLC (TA).

Statistical Methods and Hypothesis: $H_0$1: E2 and TA are not detectable in nauplii at any sampling time. This part of the study is descriptive and therefore, statistical analysis is unnecessary for testing the null hypothesis (i.e., detection of any amount of E2 or TA in nauplii will be sufficient for rejecting the null hypothesis).

Schedule: Data collection, 7/01-8/01; Technical report, 6/30/02

<u>Experiment B: Validation of Bioencapsulation Masculinization of Nile Tilapia Fry</u>
Site: Experiments will be conducted at Oregon State University, Corvallis, OR.

Methods: Oral administration of TA via bioencapsulation using Artemia nauplii in 200-l fiberglass tanks.

Laboratory and Pond Facility Fish Performance and Genetics Laboratory, OSU. 2 recirculating systems with 12 200-l tanks each. 15 females and 5 males for production of fry.

Culture Period: 4 months or until tilapias reach sampling size.

Stocking Density: 100 fry/tank

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

Nutrient Inputs: None

Water Management: Water will be maintained at 28°C, 25% water exchange once a week.

Sampling Schedule: The experiment consists of four treatments:
- fry fed bioencapsulated TA for 5 days
- fry fed bioencapsulated TA for 10 days
- fry fed bioencapsulated TA for 20 days
- fry fed bioencapsulated TA for 28 days

Control treatment will be fed control food.

Each treatment will be triplicated. At the end of 4-month grow-out period, a subsample of the tilapias in each experimental unit (50) will be killed with an overdose of anesthetic (MS-222) to determine if the treatment with TA resulted in masculinization. The following water quality parameters will be measured daily: pH, DO, and temperature.

Statistical Methods and Hypothesis: $H_0$1: Administration of TA to tilapia fry results in treatments with the same sex ratios as those of the controls. The efficacy of TA treatment will be tested comparing between treatments by Chi-squared test.

Schedule: Data collection, 9/01-7/02; Technical report, 3/30/03

<u>Experiment C: Feminization of Tropical Gar Fry</u>
Site: Experiments will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Oral administration of E2 via bioencapsulation using Artemia nauplii in fiberglass tanks (1 m^3); and immersion in E2

Laboratory and Pond Facility: Universidad Juárez Autónoma de Tabasco; 20 50-l aquaria, 3 reproduction concrete tanks (8 m^2), 50 net cages (1 m^3) for grow-out, 3 grow-out ponds (200 m^3), a total of 10 females and 30 males for production of fry.

Culture Period: 6 months (gars) or until fish reach sampling size

Stocking rate: 50 fry/tank

Test Species: Tropical gar (*Atractosteus tropicus*)

Nutrient Inputs: None

Water Management: 50% water exchange will be performed twice a week.

Sampling Schedule: For each species, the experiment will consist of seven treatments:
- fry fed bioencapsulated E2 for 5 days starting at onset of feeding
- fry fed bioencapsulated E2 for 10 days starting at onset of feeding
- fry fed bioencapsulated E2 for 20 days starting at onset of feeding
- fry fed bioencapsulated E2 for 28 days starting at onset of feeding
- fry immersed for three consecutive days in 500 µg/L E2 for 3 hrs at 50% hatching time
- fry immersed for three consecutive days in 500 µg/L E2 for 6 hrs at 50% hatching time

Control treatment will be fed control food in regular water.

Each treatment will be triplicated. At the end of 6 month grow-out period, gars will be killed with an overdose of anesthetic (MS-222) to determine if the treatment with E2 resulted in feminization. The following water quality parameters will be measured daily: pH, DO, and temperature.

Statistical Methods and Hypothesis: $H_0$1: Administration of E2 to gar fry results in treatments with the same sex ratios as those obtained in the control treatment. The efficacy of E2 treatment will be tested comparing between treatments by Chi-squared test.

Schedule: Data collection, 9/01-5/02; Technical report, 6/30/02

<u>Experiment D: Masculinization of Castarrica Fry</u>
Site: Experiments will be conducted at the Laboratory of Aquaculture at UJAT, Tabasco, México.

Methods: Oral administration of TA via bioencapsulation using Artemia nauplii in fiberglass tanks (1 m^3) and immersion in TA.

Laboratory and Pond Facility: Universidad Juárez Autónoma de Tabasco; 20 50-l aquaria, 3 reproduction concrete tanks (8 m^2), 50 net cages (1 m^3) for grow-out, 3 grow-out ponds (200 m^3).

Culture Period: 4 months or until castarricas reach sampling size.

Stocking Density: 50 fry/tank

Test Species: Mojarra castarrica (*Cichlasoma urophthalmus*).

Nutrient Inputs: None

Water Management: 50% water exchange will be performed twice a week.

Sampling Schedule: The experiment consists of six treatments:

- fry fed bioencapsulated TA for 5 days starting at onset of feeding
- fry fed bioencapsulated TA for 10 days starting at onset of feeding
- fry fed bioencapsulated TA for 20 days starting at onset of feeding
- fry fed bioencapsulated E2 for 28 days starting at onset of feeding
- fry immersed for three consecutive days in 500 µg/L E2 for 3 hrs at 50% hatching time
- fry immersed for three consecutive days in 500 µg/L E2 for 6 hrs at 50% hatching time

Control treatment will be fed control food in regular water.

Each treatment will be triplicated. At the end of 4-month grow-out period, castarricas will be killed with an overdose of anesthetic (MS-222) to determine if the treatment with TA resulted in masculinization. The following water quality parameters will be measured daily: pH, DO, and temperature.

Statistical Methods and Hypothesis: $H_0 1$: Administration of TA to castarrica fry results in treatments with the same sex ratios as those obtained in the controls. The efficacy of TA treatment will be tested comparing between treatments by Chi-squared test.

Schedule: Data collection, 7/02-2/03; Technical report, 3/30/03

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On-Station and On-Farm Trials of Different Fertilization Regimes Used in Bangladesh

Appropriate Technology Research 4 (10ATR4)/Study and Activity/Bangladesh and Thailand

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Objectives

1) Evaluate the different fertilization regimes currently used for aquaculture in Bangladesh.
2) Compare effects of different fertilization regimes on fish production, water quality, pond effluents and economic returns.
3) Recommend best fertilization regimes to small-scale rural farmers.

Significance

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

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

Aquaculture is commonly practiced using polyculture of 4-7 species of Indian and Chinese carps in manured and/or fertilized ponds (Wahab et al., 1991). In spite of extensive research has been conducted on fertilization in carp polyculture ponds in many parts of the world, such information in Bangladesh is rather scanty (Haq et al., 1993). Fish production is quite low in Bangladesh, averaging 2,800 kg ha^{-1} yr^{-1} (DOF, 1999). In rural aquaculture ponds, fish production is often lower than 1,500 kg ha^{-1} yr^{-1}. NGOs have been working with farmers to increase fish production, however, different NGOs recommend different fertilization regimes to farmers, and these regimes do not all seem to increase yields. Fertilization regimes should vary with different local conditions such as soil and source water. In some cases, the same farmers receive very different recommendations on fertilization regimes from different extension partners. Both over- and under-fertilization may cause adverse effects on fish production, water quality, pond effluents and economic returns. It is necessary to evaluate fertilization regimes and recommend appropriate fertilization strategies to farmers in order to maximize fish production, maintain good water quality, reduce environmental impact and maximize economic returns.

Anticipated Benefits

The results of this study will provide an appropriate fertilization strategy to polyculture Indian carps and Chinese carps for small-scale rural farmers. It will benefit fish culturists in Bangladesh and other countries in the region where carps are commonly polycultured.

Research Design

This study would consist of two main components, i.e., on-station trials and on-farm trials.

On-Station Trials

Location: Bangladesh Agricultural University (BAU), Field Laboratory, Mymensingh, Bangladesh (on-station trials)

Methods: Pond research

Pond Facility: Fifteen 100-m^2 earthen ponds will be used.

Culture Period: 150 days

Stocking Density: 1 fish/m^2; stocking size: 8-10 g

Test Species: silver carp *Hypophthalmichthys molitrix*, mrigal *Cirrhinus mrigala*, rohu *Labeo rohita*, catla *Catla catla*, grass carp *Ctenopharyngodon idella*, and common carp *Cyprinus carpio*; species ratio: 6:5:4:2:2:1.

Nutrient Inputs: Varying with treatment.

Water Management: Maintain at 1.0-1.2 m depth.

Sampling Schedule

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

Fish Growth: Monthly growth and total harvests. Pond effluents will be sampled at harvest. Partial budgets will be estimated to assess costs and value of fish crops.

Statistical Design, Null Hypothesis, Statistical Analysis: Experimental design: The experiment design is a randomized complete block design (due to different size of pond surface area). There will be five fertilization regimes with three replicates each. (A) PROSHIKA fertilization regime: weekly application of 1,250 kg cow manure, 15 kg urea and 22.5 kg TSP per hectare (personal communication). (B) BRAC fertilization regime: weekly application of 156 kg cow manure, 27.5 kg urea and 13.1 kg TSP per hectare (personal communication). (C) CARITAS fertilization regimes: fortnight application of 1,500 kg cow manure per hectare. (D) BAU fertilization regime: fortnight application of 5,000 kg cow manure, 50 kg urea and 50 kg TSP per hectare (Wahab et al. 1999). (E) CRSP organic fertilization regime developed from Nile tilapia ponds: 250 kg cow dung (DM) ha^{-1} wk^{-1} supplemented with urea and TSP to give 28 kg N and 7 kg P ha-1 wk-1 (Knud-Hansen et al. 1991a).

Null hypothesis: Different fertilization regimes in carp polyculture ponds have no effect on the growth, water quality and pond effluents.

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

On-Farm Trials

Location: Working sites of four NGOs (PROSHIKA, BRAC, CARE, and CARITAS), Bangladesh (on-farm trials)

Methods: Pond research

Pond Facility: 6 earthen ponds in each of four NGO sites will be used.

Culture Period: 180 days

Stocking Density: 1 fish/m^2; stocking size: 8-10 g

Test Species: silver carp *Hypophthalmichthys molitrix*, mrigal *Cirrhinus mrigala*, rohu *Labeo rohita*, catla *Catla catla*, grass carp *Ctenopharyngodon idella*, and common carp *Cyprinus carpio*; species ratio: 6:5:4:2:2:1.

Nutrient Inputs: Varying with different treatments

Water Management: Maintain at 1.0 to 1.2 m depth

Sampling Schedules: All water quality parameters mentioned before will be analyzed at beginning and end of the experiment. Fish growth at final harvest. Partial budgets will be estimated to assess costs and value of fish crops.

Statistical Design, Null Hypothesis, Statistical Analysis

Experimental design: The experiment is a randomized complete block design. In each NGO site, the best fertilization regime from on-station trial will be compared with the respective NGO fertilization regime, three replicates each.

Null hypothesis: Different fertilization regimes in carp polyculture ponds have no effect on the growth. The results of fish growth and water quality will be analyzed for significant differences between treatments using ANOVA.

Regional Integration

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

Schedule

July to December 2001 and June to December 2002

Report submission: March 2002 for on-station trials and March 2003 for on-farm trials

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Use of Clinoptilolite Zeolites for Ammonia-N Transfer and Retention in Integrated Aquaculture Systems and for Improving Pond Water Quality before Discharge

Appropriate Technology Research 5 (10ATR5)/Experiment/Thailand

Collaborating Institutions
Asian Institute of Technology
Amrit Bart

Michigan State University
Ted R. Batterson
Donald L. Garling
Christopher F. Knud-Hansen

Objectives
The ultimate goal of this proposed research is to adapt existing technologies using natural clinoptilolite zeolites to provide a more socially acceptable and efficient way to integrate animal manures in pond fertilization, conserve and recycle on-farm resources, and lessen environmental impacts.

Fertilization Efficiency Objectives
1) Determine the relationship between ammonia absorption/saturation by clinoptilolite from fresh swine and liquefied chicken manures versus exposure time to the manures.
2) Determine the rate of release of ammonia from ammonia-enriched clinoptilolite when used as a nitrogen fertilizer for stimulating natural food production in an outdoor aquaculture system.
3) Determine the ability of clinoptilolite to moderate ammonia concentrations in a fertilized outdoor culture system.

Pond Water Discharge Objectives
1) Evaluate the effectiveness of clinoptilolite for removing nitrogen and phosphorus from pond discharge.
2) Evaluate the potential utility of nutrients reclaimed by clinoptilolite for recycling in pond fertilization.

Significance
Natural zeolites are aluminosilicate minerals found in volcanogenic sedimentary rocks worldwide (Mumpton, 1999). Natural zeolites possess several important properties including adsorption, cation-exchange, dehydration-rehydration, and catalysis. Considerable scientific research in the last few decades has identified broad applications for natural zeolites in construction materials, soil improvements for water and nutrient retention, treatment of water and wastewater for removal of heavy metals and nutrients, dietary supplements for farm-raised animals, health care, and other beneficial uses (Mumpton, 1999).

Clinoptilolite zeolites, $(Na_3K_3)(Al_6Si_{30}O_{72})\bullet 24H_2O$, are one of the 40+ types of naturally existing zeolites. Clinoptilolites possess a cation-exchange capability of about 2.25 meq g^{-1}, and are able to exchange ammonium-N with sodium (Na) and potassium (K) (Mumpton, 1999). One gram of clinoptilolite can take in about 2.2 mg ammonium-N. This cation-exchange capability has been utilized effectively for terrestrial agriculture, where clinoptilolites are first saturated with ammonium-N and then incorporated into crop soils. In this way they act as a slow-release fertilizer, with plants able to extract the sequestered ammonia from the clinoptilolite (Barbarick and Pirela, 1984; Lewis et al., 1984; Dwairi, 1998). Not only does clinoptilolite improve nitrogen fertilization efficiencies, it also reduces nitrate leaching by inhibiting the nitrification of ammonium to nitrate (Perrin et al., 1998). Most of the manure-ammonia sequestered in the zeolite is unavailable to nitrifying bacteria because of the small (4-5 angstrom) pore size of the crystal lattice structure (Mumpton, 1999). Furthermore, clinoptilolites are also used for animal waste management. Clinoptilolites are replacing clays in the cat litter market, and are being used to create an odorless, nitrogen-rich compost from farm livestock manures.

The use of clinoptilolites in aquaculture has focused on ammonia removal for the aquarium industry and freshwater culture systems (Bower and Turner, 1982; Dryden and Weatherley, 1987). The research below, however, proposes an analogous use of clinoptilolite for aquaculture as currently used for terrestrial agriculture and animal waste management: i.e., as a vehicle for ammonia absorption and subsequent fertilization to stimulate algal productivity.

Applying clinoptilolite technologies for livestock-fish integrated systems should improve sustainability by increasing nutrient utilization efficiencies while reducing undesirable farm outputs. Most of the nitrogen entering a farm as animal feeds ends up as ammonia in manure, which is either volatilized creating noxious odors or leached out as nitrate. By capturing this ammonia-N before it gets either volatilized or nitrified, and using that nitrogen to promote algal productivity in ponds, the farmer not only improves the farm environment by reducing noxious odors and nitrate leaching, but recycles an otherwise lost nutrient for increased farm productivity. Incorporating clinoptilolite with fresh animal manures may also improve the social acceptability of integrated aquaculture.

Furthermore, by transferring ammonia from animal manures to clinoptilolite, and then applying the ammonia-enriched clinoptilolite to ponds, the farmer can fertilize ponds with manure-N without adding additional BOD (biochemical oxygen demand). The major environmental risk of adding manure to ponds is the creation of anoxic conditions in the water. Research clearly shows that both algal and fish productivity can be quite high in ponds without the risk of pond water deoxygenation if no additional organic matter is added (Knud-Hansen et al., 1993). Using clinoptilolite to transfer manure ammonia turns animal manure into a source of inorganic nitrogen, and should eliminate associated risks of adding manures to ponds.

Clinoptilolites are also increasingly being used for wastewater treatment (Holman and Hopping, 1980; Ciambelli et al., 1985). For example, 18 municipal wastewater treatment facilities in Brisbane and other cities in Australia use zeolites for ammonia removal and for the flocculation, settling, and removal of phosphates in domestic wastewater (Oláh et al., 1989; Charuckyj, 1997). The research proposed below is a simple application of this existing clinoptilolite technology for cleaning pond water before being discharged into streams and canals. By removing soluble nitrogen and phosphorus before discharge, receiving waters are at less risk of eutrophication. By capturing these nutrients, they can be recycled back into ponds for stimulating algal productivity. Nutrients which would otherwise be lost from the farming system and degrade surrounding environments are instead recycled to increase farm productivity. Furthermore, clinoptilolites are renewable, since regeneration can be simply accomplished through heating or immersion in a salt solution. And since clinoptilolites are natural, inert, do not degrade, and even used in animal feeds (Pond and Yen 1984), they have no associated environmental risks.

Quantified Anticipated Benefits

Anticipated benefits are discussed generally above in the "Significance" section. More specifically, anticipated benefits to the farmer will be:

1) Economic savings with reduced need for purchasing additional fertilizers through the retention and recycling of nutrients on farm;
2) Improved quality of life with reduction of noxious odors from animal manures;
3) Reduced risk of deoxygenation of ponds when integrating animal manures with pond aquaculture; and
4) Economic savings if there are legal liabilities associated with downstream impacts from pond water discharges, particularly from intensive aquaculture systems.

And to the community:

1) Improved quality of life and social acceptability with the reduction of noxious odors from animal manures and

2) Reduced risk of environmental contamination and downstream eutrophication from ammonia volatilization, nutrient leaching, and pond water discharge.

Research Design

All research will take place at the Asian Institute of Technology (AIT), Thailand, within their Agriculture, Aquatic Systems and Engineering Program. Mr. Yuan Derun, a doctoral student within the graduate program, will be responsible for conducting the research under the direction of Dr. Knud-Hansen. The clinoptilolite to be used in all studies originates from Potosí, Mexico, and has an exchangeable K:Na ratio of about 8:1. Statistical analyses will include Analysis of Variance (ANOVA) for comparison of treatment means, and correlation and regression analyses for identifying relationships. The economic viabilities of the proposed clinoptilolite technologies for rural farming systems will also be evaluated as part of the overall research analysis.

Standard cost-benefit analysis at the farm level: costs include materials, time, labor, alternative sources of pond nutrients (i.e., fertilizers and manures), etc.; benefits include savings on fertilizer costs by recycling nutrients otherwise lost to the farming system, perhaps increased marketability of fish not raised directly on animal manures, etc. Economic analysis will focus on the potential for zeolite technology as applied for rural integrated farms in Thailand. Exact details will develop as the technology evolves through the proposed research.

1) Relationship between Clinoptilolite and Ammonia-N Absorption from Animal Manures

This relationship will be examined through a bench study. Crushed clinoptilolite (about 1-2 mm diameter grain size) will be contained in plastic mesh bags at approximately 1 kg clinoptilolite per bag, and immersed in buckets containing fresh swine and chicken manure. Water will be added as necessary to make the manure solution more liquid, which will facilitate the cation-exchange process between K and ammonium. The primary variable examined will be the length of time required to saturate the clinoptilolite with ammonium ions. Additional variables will be the effect of agitating the clinoptilolite bags on the speed of ammonia absorption, and calculating weight to weight relationships between the amount of clinoptilolite required versus the quantity and types of manure. Analytical measurements will be total Kjeldahl nitrogen of the manures before and after exposure to clinoptilolite, and ammonia-N retained in the clinoptilolite, which can be extracted through persulfate digestion.

2) Release of Ammonia from Ammonia-Enriched Clinoptilolite in a Fertilized Pond

The release of ammonia from clinoptilolite enriched with manure-ammonia will be examined both in a bench study and in an outdoor tank experiment. The bench study will be a preliminary evaluation of the ability of a mixed algal culture to extract ammonia from ammonia-enriched clinoptilolite, and examine the relationships between per cent ammonia saturation of clinoptilolite and algal biomass/productivity. The mixed algal culture will come from a fertilized pond and placed in 20-L buckets. Triple superphosphate (TSP) will be added to make algal productivity in the cultures N-limited. Clinoptilolite with a full range of percent ammonia saturation will be added to the containers. There will be a total of 10 different saturation levels in triplicate containers, with algae cultured outdoors for one week. Algal biomass will be determined daily from chlorophyll *a* measurements made with a hand-held fluorometer. Algal productivity will be determined by differences in dissolved oxygen measured by a hand-held dissolved oxygen meter measured at pre-dawn and mid-day. Ammonia-N will be measured in the clinoptilolite before and after the culture period, and in each container daily at mid-day.

Based on the results from the two bench studies described above, an 8-week grow-out experiment will be conducted in 2.5 m x 2.5 m x 1 m outdoor concrete tanks located at AIT. Nile tilapia fingerlings, about 10 g fish^{-1}, will be stocked at 3 fish m^{-2}. There will be a total of 13 treatments, with three replicate tanks (experimental units) per treatment assigned randomly in a completely randomized design. Nine of the treatments will examine the transfer of ammonia-N from zeolite into tank water. There will be three different amounts of clinoptilolite enriched with ammonia from three different sources: swine manure, chicken manure, and concentrated solution of urea. The ammonia-enriched zeolite will be in plastic mesh bags (about 1 kg zeolite/bag), with the three different numbers of bags per treatment. With all of the above nine treatments, bags will be replaced weekly. The actual number and size of bags will be determined based on results from the preliminary bench studies described above.

TSP will be added to all tanks at about 1.0 g TSP-P m^{-2} wk^{-1} to provide enough soluble P to prevent P-limitation of algal productivity. The last four treatments will be a dose-response evaluation of clinoptilolite's ability to moderate ammonia concentrations in culture water. One treatment will have no bags of clinoptilolite, while the other three will have increasing numbers of bags. All four treatments will be fertilized with urea at 3.0 g urea-N m^{-2} wk^{-1} and 1.0 g TSP-P m^{-2} wk^{-1}. These fertilization rates correspond to rates established by previous MSU/AIT research found to be very productive without excessive fertilization. The treatment without any bags will serve as the control for the other 12 treatments. Changes in algal biomass will be monitored weekly with a hand-held fluorometer which measures chlorophyll *a*, net algal productivities will be monitored weekly by diel changes in dissolved oxygen, tilapia growth will be measured at the start and end of the grow-out experiment by length and weight measurements. Mid-afternoon water temperatures, turbidities, and ammonia-N will be monitored weekly in all 39 tanks.

3) <u>Reclamation of Nutrients from Pond Water Discharge</u>
Twenty tanks used in the grow-out experiment will be selected for their wide range of chlorophyll *a* and ammonia-N concentrations, and drained through clinoptilolite filters. Twenty clinoptilolite flow-through filters will be made from the 20-L buckets used in the bench studies. Assuming that 1 g of clinoptilolite can remove 2 mg of ammonia-N, then a tank with about 1 mg L^{-1} ammonia-N would require approximately 3 kg clinoptilolite. All 20 filters will contain identical quantities of clinoptilolite, the actual amount to be based on results of the preliminary bench studies and actual ammonia-N concentrations. The two factors will be the addition of clinoptilolite powder (CP, about 0.8 mm) to 10 tanks to flocculate P before going through the filter, and either "slow" or "fast" discharge flow rates. Actual pump rates will be determined on site. Therefore, this will be 2×2 factorial designed experiment, with four treatments (i.e., CP-slow, no CP-slow, CP-fast, no CP-fast), with five replicates per treatment. Filter efficiencies will be determined by measurements of total P, soluble P, ammonia-N, nitrate-nitrite-N, and Kjeldahl-N before and after filtration.

<u>Regional Integrations</u>
AIT also has a well-established Training and Consultancy Unit which gives regional workshops on various aspects of aquaculture production systems. The knowledge generated from the proposed research can be readily incorporated into the appropriate workshop(s). There will also be five regional workshops on using pond dynamics to promote sustainable aquaculture included as a separate activity in this proposal. In addition to AIT, the other workshop locations will be at aquaculture research institutes and stations located at Bangladesh, Cambodia, Laos, and Vietnam where AIT and the PD/A CRSP have established formal relationships. Information generated from the above research will be incorporated into these proposed workshops. Strengthening ties between these countries and AIT and the PD/A CRSP is an important component of the Regional Plan For Southeast Asia.

<u>Schedule</u>
All proposed research is scheduled to take place between January and May 2002. Knud-Hansen will make two trips to AIT, of approximately three weeks each (excluding periods away from AIT to give workshops). During the first trip the bench studies on ammonia absorption by clinoptilolite and subsequent release into culture water will take place, and the grow-out study initiated. During the second trip the grow-out study will be completed and the nutrient reclamation study on pond water discharge will be conducted. Final report will be submitted no later than 31 July 2002.

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A Study of Aquaculture Brownfields: Abandoned and Converted Shrimp Ponds in Thailand

GIS: Planning, Policy, and Global Data Analysis Research 1 (10GISR1)/Study/Thailand

Collaborating Institutions
Asian Institute of Technology, Thailand
Amrit Bart

The University of Michigan
Steven R. Brechin
James S. Diana

Objectives

1) Determine the current state of abandoned and converted shrimp ponds in the study area.
2) Assess attitudes, concerns, and interests of a number of stakeholders, such as farmers, government personnel, community and business leaders, etc., about abandoned ponds and possible alternative uses.
3) Assess the social and technical conditions necessary for diffusion and adoption potential alternative uses.

Significance

Abandoned shrimp ponds mar the landscape of Thailand as well as other countries. They are a rural equivalent to "brownfields," the abandoned and degraded industrial sites in urban centers. Both represent environmental degradation, lost economic opportunity, and underutilized land. This proposal attempts to explore the status of abandoned shrimp ponds and their social and technical potential for rehabilitation and reuse.

Shrimp culture has been variously considered as a panacea for aquaculture and economic development (New, 1997), or a major environmental catastrophe (Goldburg and Triplett, 1997). Shrimp culture has expanded dramatically since 1985, resulting in annual increases of 25% or more in yield worldwide (Diana, 1993; New, 1997). Some view this increase as problematic, citing increased pollution from effluents, conversion of lands to shrimp ponds (especially mangrove ecosystems), and disease introduction (Goldburg and Triplett, 1997). Others question the actual levels of conversion from mangrove to shrimp ponds and believe that mangroves were already being degraded long before shrimp farming became popular (Menasveta, 1997; Fast and Mensaveta, 2000). Clearly, public perception is that shrimp farming is environmentally destructive and that control needs to be exerted.

One major damage caused by shrimp farming is the use of ponds for a short time then abandonment because of disease, pollution of local waters, or economic failure. These abandoned ponds, often miles from the coast, cannot be easily reclaimed because the soils have become saline by input and evaporation of salt water in the culture system. The magnitude of the problem of abandoned shrimp farms in Thailand is unknown. It appears as though it could be a large problem getting larger. The estimates focus on particular areas, but no aggregate, regional estimates have been possible. Briggs and Funge Smith (1994, as referenced in Stevenson, 1997) estimate an area of 40,000 to 45,000 hectares south of Bangkok were abandoned when shrimp production fell in 1989. Another report, produced by NACA, estimates that 22% of farms in the Samut Sakhon province were abandoned at that time. And another, focusing on Prachaup Khiri Khan province estimates abandonment of 70-80% of the farms (Stevenson, 1997).

The problem of shrimp farm abandonment, leaving land unfertile in need of rehabilitation may worsen in coming years. It is widely believed that, as currently practiced, shrimp culture poses serious environmental and public health threats (Fegan, 1996; Stevenson, 1997; Flaherty et al., 1999). Flaherty et al. assert that small-scale shrimp farming is economically as well as environmentally unsustainable and suggest that the lands currently involved in production will be abandoned and left in a state unsuitable for other agricultural pursuits.

In some cases, culturing of brackishwater fish, using freshwater as the input and allowing salts to dissolve from the sediments, has occurred in such ponds. Previous CRSP work on reclaiming soils with acid sulfate conditions (Lin, 1986) and current CRSP work on fertilization of brackish water for tilapia production (Ninth Work Plan Thailand Research) provide basic concepts for reclamation of abandoned shrimp ponds in Thailand. Over time, we believe that such systems will not only produce a cash crop, but will also result in fertilization of the pond soils by fish waste and reduction in salinity by dissolution from fresh water, then discharge on harvest. Such processes may reclaim the area for other aquaculture or agriculture purposes. However, for this to be feasible, we need to use systems that will be successful in producing economic returns for culture in abandoned shrimp ponds.

In addition to environmental and economic concerns, the issue of shrimp culture has been controversial on social grounds. Rice cultivation has been the root of Thai culture for centuries (Flaherty et al., 1999) and it is threatened to be displaced by inland shrimp cultivation. Shrimp farms have moved into the interior of Thailand and, because of declining productivity, they require increasing amounts of land. This land is obtained by inundating rice paddies with salt water that is trucked in from the coast. There are conflicts between practitioners of both types of agriculture for water use, and because of salt infiltration from shrimp farms that have reduced rice yields. Rice farmers in inland areas are more vocal in their opposition to shrimp culture than coastal farmers that have suffered for decades.

This study would evaluate the current status of shrimp ponds in three study sites and to determine the level of abandonment, as well as social, legal, economic, and technical constraints to their reclamation. The majority of research into the environmental and socioeconomic costs of shrimp culture has been conducted primarily in response to mangrove deforestation. There is a need to address the unique concerns of aquaculturists in other areas including inland zones.

This study consists of two main components. The first is to determine current state of shrimp pond abandonment and potential reuse in our study areas. The second is to assess the social acceptance and technical feasibility of potential alternative uses of abandoned ponds as well as the conditions necessary for their adoption and diffusion by culturists. On assessing current status, the researchers would attempt to answer a number of questions including, how many shrimp ponds have been abandoned and why? Are owners considering other uses for their abandoned or poorly functioning ponds? If so, what might those potential uses be? Have they already found creative ways to rehabilitate ponds for other uses, such as for fish or other type of agricultural production? Or are they struggling to identify alternatives? Are the ponds still in ownership of the original shrimp farmers or are they public lands or land available for sale? The second component of the research would focus on the social acceptance and technical feasibility of identified alternatives and the potential for new and unexplored possibilities. Here research questions would include, what barriers keep users from fully implementing identified alternatives? Are there financial, technical, informational, policy, marketing, or other types of constraints? Are there other alternatives that have not been fully explored? If so, what are they? And why have they not been explored? How might these other innovations be developed technically and diffused among potential adopters? What adoption package would likely be the most successful given social, economic, cultural, and technical variables?

Anticipated Benefits

The problems with abandoned shrimp ponds are at least partly perceptional and partly reality. This study would quantify the extent of the problem and identify technical, social, legal, and economic constraints to its reversal. Sustainable aquaculture is highly debated, but at least it should involve continued reuse of pond systems. Therefore, this study will lead to better understanding of sustainable aquaculture in the region.

Abandoned shrimp ponds represent environmental degradation, loss economic opportunity, and underutilized land. The results from this study could also eventually lead to the development of ecologically and economically effective alternative systems for fish (or other agricultural production) in brackish water. It would help to better understand the local constraints to aquaculture sufficiently to adapt the production system to transportation, markets, alternative forms of employment, and to overcome social system constraints.

Research Design

For the study's first component, researchers will develop a GIS database of existing abandoned shrimp ponds in three study locations representing sites near major cities and more rural areas. The sites for analysis include Samut Sakhon, a major shrimp zone along the central coast; Chacheng Sao, an area near Bangkok with urbanization and saltwater intrusion; and Kanachanaburi, an area with extensive pond development on reclaimed mangrove systems. We will use remote images to determine the number of ponds in about 30 km of coastline and within 10 km of the shore. We will then do ground truthing to determine the fraction of those ponds still in operation, as well as those converted to other uses or abandoned. For existing ponds (in use or abandoned), surveys will be done to determine basic characteristics of ownership (e.g., farmer, local or foreign corporation, local or national business association/ partnerships, community, other), number of ponds, pond size, socioeconomic characteristics of location and community, and technical information about conditions of the pond. These variables will all be attributes of the GIS database to allow evaluation of areas of active and abandoned ponds, selection of appropriate survey sites, and the geographic and political extent of the problem. The GIS analysis will involve both laboratories at AIT and the Spatial Analysis Lab at UM. Both have well developed GIS support systems.

For the study's second component, researchers will assess the social acceptance and technical feasibility of potential alternative uses of shrimp ponds. This information will be acquired through face-to-face, semi-structured interviews with shrimp pond owners, whether local farmers, business leaders, and so on. Similar type interviews will be conducted with related professionals, such as government agency personnel, the staff of environmental and developmental non-government organizations, and university faculty and researchers familiar with shrimp pond activities and aquaculture.

Regional Integration

This research would provide useful information for other countries in the region that face similar problems with abandoned shrimp ponds. Use of GIS databases, and the potential for determining ecological factors affecting use and abandonment of shrimp ponds, may allow regional managers to have means of remotely assessing and managing their shrimp culture resources. Alternative uses to ponds should also be appropriate elsewhere. This research may provide impetus in this area by encouraging others to pursue similar studies and by actually identifying alternative uses for ponds.

Schedule

GIS and field work: September 2001 to February 2003
Report submission: April 2003

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Assessing Watershed Ponds for Aquaculture Development in Thai Nguyen, Vietnam

GIS: Planning, Policy, and Global Data Analysis Research 2 (10GISR2)/Study/Thailand and Vietnam

Collaborating Institutions

Asian Institute of Technology, Thailand
C. Kwei Lin
Yang Yi

Research Institute for Aquaculture No. 1, Vietnam
Le Thanh Luu

The University of Michigan
James S. Diana

Objectives

1) Conduct survey on biophysical features, land and water uses, and socioeconomic conditions of watershed areas in Thai Nguyen.
2) Develop a detailed GIS database for planning of aquaculture development in the study area.
3) Identify and estimate suitable watershed ponds for aquaculture.

Significance

A large number of watershed ponds and reservoirs were created mostly for household water supply and crop irrigation in mountainous areas in central and northern Vietnam. Occasionally, these ponds are also used for fish culture. Thai Nguyen district, situated approximately 100 km north of Hanoi, is a population center serving as a gateway to northern Vietnam. The area possesses numerous small reservoirs and watershed ponds used for irrigating tea plantations, rice fields and fruit orchards; some are also used for aquaculture. To most inhabitants in mountainous areas in northern Vietnam, supply of animal protein is relatively limited, including small contributions from fish. Recently, the Vietnamese government has initiated aquaculture development in those areas as a means to increase protein availability. Therefore, the development of aquaculture in Thai Nguyen will also serve as a model for watersheds in other provinces.

In recent years, surveys have been conducted in Thai Nguyen to evaluate biological, physical, social and economic conditions for aquaculture development. However, most lack systematic spatial data. Planning activities to promote aquaculture in Thai Nguyen need spatial analysis because of geographical variation in biophysical features and socio-economic status (Kapetsky et al., 1987; Nath et al., 2000). This project proposes to develop a GIS database of watershed ponds, coupled with other physical, biological, and social attributes of northern Vietnam. Development of a GIS database is a first step in formally evaluating the effects of local geographic and social conditions on the production of fish in watershed ponds.

With an adequate database, Geographic Information Systems (GIS) can serve as a powerful analytic and decision-making tool for aquaculture development. Furthermore, it can also be used for management and to test consequences of development (Aguilar-Manjarrez and Ross, 1995). GIS systems are a strong component of research at both University of Michigan and the Asian Institute of Technology. These systems will be available and applied to aquaculture development in northern Vietnam.

Anticipated Benefits

This study will provide objective means for planners to determine potential aquaculture development in Thai Nguyen province. The results will provide governmental agencies, the private sector, and non-governmental organizations involved in aquaculture development. It also will establish a model case in planning for watershed aquaculture development in other parts of Vietnam.

Research Design
This study consists of two components. The first component is a survey to assess the current state of aquaculture systems in watershed ponds, and the second is the data to develop a GIS database and identify suitable watershed ponds for aquaculture.

Location: Thai Nguyen province, 100 km from Hanoi; Research Institute for Aquaculture No. 1, Vietnam

Research Methods
Survey
Specific Objectives
- To describe different aquaculture practices in term of species, production and management in different farming systems (VAC system– garden-pond-livestock; rice-fish system; cage culture; pond culture)
- To identify the importance of socio-economic and environmental factors in aquaculture (infrastructure, regulation, water availability, soil type, soil pH, and water quality)
- To identify the attitude of farmers to watershed ponds and aquaculture (the importance of watershed ponds for irrigation, domestic use or aquaculture; the importance of aquaculture in household economy)

Data Collection and Analyses
Secondary Data Collection: Socio-economic conditions have a strong interaction with aquaculture. Data about socio-economic conditions in study areas will be collected at the Department of Statistics of Thai Nguyen and the district level. At the same time relevant information on aquaculture practices will also collected;

Primary Data Collection: One-hundred households will be selected for interview, and one-hundred watershed ponds for soil and water quality analyses. Pond analyses will be done monthly following standard CRSP methods. Pond selection will be based on the location and culture system. The location of the farm will be recorded by GPS coordinates for transfer to the GIS database. The interviews will focus on farmers' socio-economic status and attitudes on aquaculture as well as potential and constraints for aquaculture;

Data Analysis: Statistical methods and socio-economic methodologies will be used to analyze the data. The analytic hierarchy process (Canada et al., 1996) will be used to identify the importance of the different factors on aquaculture.

GIS Database Development
Data Collection
Remote Sensing Data: two satellite images will be obtained for dry (SPOT January 2001) and wet seasons (SPOT July 2001). Land use classification and simple overlay will be carried out to identify the potential and changing areas of watershed ponds in the dry and wet seasons;

Secondary Data: secondary data will be collected from relevant government offices. These data will be divided into three broad categories: physical and environmental characteristics (water resources, climate, temperature, soils, topography), socio-economic (land use, infrastructure, population density, income distribution), and constraints for aquaculture (protected land, polluted areas, and urban centers);

Primary Data: primary data will be gathered by the field survey that will collect additional data as well as confirm the secondary data. This survey will involve the inspection of the study area in term of hydrological investigation and land use;

Weighting and Scoring: weight and score will be based on the level of importance of the factor that will influence aquaculture. The weight for each factor will be determined by using the

Analytic Hierarchy Process (Canada et al., 1996). The score of each level of each factor will be determined from the result of the first component and input decision from experts.

Data Processing: data processing is a process of transforming spatial data and non-spatial data (attributes) into a database system using ARC/INFO software. There are several steps involved:

Digitizing (Data Input): Digitizing is the way to input the spatial data into a computer. The data, which are in the form of maps, can be digitized directly. But data in the form of tables and reports will be used to create attribute tables, and then the attribute tables will be integrated with polygon maps.

Overlay Maps: The main purpose of overlays is to find the land suitable for aquaculture. All digitized maps (physical, environmental characteristics and socio-economic status) will be overlaid with their relative attribute data so that comparable mapping units will be produced. The total score will be calculated for each comparable mapping unit as a sum of the weighted scores. Dissolved function will be used to produce suitability maps. Boolean classification will be used to overlay suitability maps with constraint maps to eliminate unsuitable areas.

Regional Integration

The results of this study will be made available to relevant governmental agencies for consideration in aquaculture development in the area. Our project counterpart is actively involved in aquaculture development in the mountainous areas in northern Vietnam.

The information and GIS database from this study will establish objective criteria for aquaculture development based on an integrated approach. This model will set an example for the Indochina region.

Schedule

August 2001 to December 2002
Report submission: March 2003

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Optimal (Profit-Maximizing) Target Markets for Small- and Medium-Scale Tilapia Farmers in Honduras and Nicaragua

Marketing and Economic Analysis Research 1 (10MEAR1)/Study/Honduras and Nicaragua

Collaborating Institutions
Escuela Agrícola Panamericana, Zamorano, Honduras
Freddy Arias
Dan Meyer

Universidad Centroamericana, Nicaragua
Agnes Saborio

University of Arkansas at Pine Bluff
Carole R. Engle

Objectives

1) Estimate marketing costs associated with transportation and storage for both live and processed fish in Honduras and Nicaragua.
2) Identify specific target markets that will maximize profits for small and medium-scale tilapia farmers in Honduras and in Nicaragua.

Significance

Identification of market segments that are most likely to purchase farm-raised tilapia does not mean that it is economically feasible for a tilapia farmer to sell his/her product to that market segment. In many countries, storage problems, transportation costs, and risks associated with marketing a highly perishable product such as tilapia prevent the development of viable domestic markets. This study will address whether the prices that market outlets are willing to pay for various sizes and product forms of tilapia by the vendors most likely to increase sales of tilapia are adequate to cover storage and transportation costs and still provide a profit for tilapia growers. The analysis will incorporate risk elements to evaluate profit-maximizing marketing strategies under conditions of risk aversion as well as for risk-tolerant growers.

Linear programming (LP) refers to the computational procedure used in the allocation of limited resources to maximize profit or minimize costs of producing a specific commodity (Shang, 1990). It has been widely used as a tool for solving resource allocation problems. While LP analysis has been used most commonly to select the best combination of inputs to maximize profits or to formulate least-cost feed rations, it can also be used to evaluate allocation of output to supply geographic markets. An innovative use of this technique is to select, from the perspective of the farmer, the most profitable geographic and demographic markets

The risk inherent in the parameters of a model can be depicted through different risk programming techniques. The intention of the risk model is to adequately represent the decision-maker's response to parameter risk (McCarl and Spreen, 1994). The ultimate goal is to generate a robust solution that yields satisfactory results across the distribution of parameter values.

LP models have been used to schedule harvesting and stocking dates for shrimp aquaculture in several Latin American countries (Perez, 1986 in Panama; Dunning, 1989 in Ecuador; Stanley, 1993 in Honduras). Stanley (1993) concluded that, assuming a constant survival rate (70%) across stocking densities and months of the year, farm managers should use the highest stocking densities considered in the model and extend the duration of the grow-out cycles. Hatch et al. (1987) demonstrated the usefulness of explicitly considering risk in the formulation of optimal farm plans for shrimp culture in Panama.

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* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

Valderrama (2000) used risk programming techniques to outline optimal plans of activities for shrimp farms in Honduras with data reflecting the impact of Taura Syndrome Virus and White Spot Syndrome Virus and evaluated various management plans related to the onset of these viral epizootics.

Mathematical programming analysis has been used to evaluate a variety of management issues on catfish farms. This technique was used to compare aeration strategies on catfish farms (Engle and Hatch 1988). Engle and Pounds (1994) later developed a two-year model to estimate costs and benefits associated with single- and multiple-batch production systems of catfish in such a model. Risk factors, various stocking densities, and marketing strategies were modeled to determine that farmers use multiple-batch strategies, even though profits are lower than with single-batch strategies, due to off-flavor and cash flow reasons. Engle et al. (1995) extended this analysis to estimate off-flavor costs on catfish farms by including farm-level off-flavor data sets. Hatch and Atwood (1988) used risk programming to analyze catfish production.

This study proposes a more innovative use of LP techniques to identify optimal marketing strategies for varying tilapia farm sizes and farm locations. No LP analyses of tilapia production in Latin America have been identified in the literature.

Quantified Anticipated Benefits

The primary direct beneficiaries of this study will be tilapia growers in Honduras and in Nicaragua. The discrete choice study described above will identify those market outlets likeliest to purchase farm-raised tilapia, but growers must be able to sell tilapia at prices that cover both production and marketing costs as well as provide a profit. Some types of market outlets may entail greater marketing or transportation costs than others. With sound guidance on the types of market outlets with the most favorable marketing margins from the perspective of the grower, the grower can make better decisions as to the best marketing strategy for his/her farm operation.

Honduras and the region will benefit secondarily from this project through the enhanced analytical ability of the Honduran student to be funded from this project. The region will also benefit from the economic growth anticipated from enhanced market development and profitability of tilapia farms. The PD/A CRSP will be a secondary beneficiary because increased market opportunities for small and medium-scale tilapia growers will result in greater impacts from the PD/A CRSP production optimization research.

Research Design

Location of Work: The majority of this work will be done at the University of Arkansas at Pine Bluff. However, additional data on storage, transportation, and other marketing costs will be collected in Honduras and in Nicaragua.

Methods: Mathematical programming models will be developed with profit-maximizing objective functions using standard techniques (Hazell and Norton, 1986; McCarl and Spreen, 1994). The models will be based on tilapia production technologies and production costs for Honduras as described in Green and Engle (2000). Different levels of tilapia production technologies will be modeled as separate activities within the model along with their associated costs and input requirements. Separate models will be developed for Honduras and for Nicaragua.

Alternative target markets will be specified as marketing activities. Appropriate transfer and balance rows will be created to link the production and marketing activities. Target markets will be defined based on results from the discrete choice study. These will include geographic markets such as the urban centers of Tegucigalpa and San Pedro Sula in Honduras and Managua in Nicaragua as well as smaller towns such as those included in the surveys described in the first study. The geographic markets modeled will be segmented into market channel categories of restaurants, supermarkets, and open-air fish markets. Each of these will be further subdivided into categories and classifications developed with results from the previously described study. These categories and classifications will be based on those independent variables found to be significant in the discrete choice analyses. They may include categories such as the size of the supermarket or restaurant, whether it is a chain or an independent, the type of clientele served, cuisine types, etc. The product form, product size, volume requirements, delivery quotas required, and other key factors as determined from the survey data will be specified as constraint equations in the model with appropriate technical coefficients developed from the survey data.

Additional data will be collected in Honduras and Nicaragua on storage costs, transportation costs, ice, labor, and other marketing costs. Direct personal interviews will be conducted with wholesalers identified in the survey data as principal suppliers of fish and seafood. Costs associated with the storage and transportation of a wide variety of volumes of fish, both live and on ice, will be determined. These marketing costs will be entered into the model as marketing cost constraint equations across the different marketing strategies and production activities.

The model will be solved for a variety of farm sizes to determine which marketing strategies maximize profits. Other versions of the model will simulate farms located in different regions of the country. Once the base solutions have been identified for the various farm scenarios identified, risk constraints will be added to the model using Target MOTAD techniques (Hazell and Norton, 1986; McCarl and Spreen, 1994). The primary source of risk to be analyzed in this study will be marketing risks associated with storage problems, transportation problems, power outages, and price risk.

Regional Integration

The Regional Plan for Central America refers specifically to planning and implementing economics and marketing research activities in the region. Research needs for tilapia culture specifically refer to economic analysis and market development.

Schedule

7/1/01	Initiate project
8/31/01	Collect storage, transportation, and marketing cost data in Honduras and Nicaragua
12/31/01	Have prototype model developed for validation.
6/30/02	Have completed models and analyses for Honduran tilapia farms.
12/31/02	Have completed models and analyses for Nicaraguan tilapia farms.
4/30/03	Submit final report and have completed manuscripts and articles on the project.

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Development and Evaluation of a Simple Market Feasibility Assessment Methodology

Marketing and Economic Analysis Research 2 (10MEAR2)/Study/Mexico (A) and Peru (B)

Collaborating Institutions
Instituto de Investigaciones de la Amazonia Peruana, Peru
Fernando Alcántara Bocanegra

Universidad Juárez Autónoma de Tabasco, Mexico
Wilfrido Contreras-Sánchez

University of Arkansas at Pine Bluff
Carole R. Engle

Objectives

1) Develop a methodology for rapid assessment of market feasibility for aquaculture species.
2) Evaluate the methodology developed for rapid assessment of market feasibility for aquaculture species.

Significance

Comprehensive marketing studies are very expensive and resource intensive. They require a great deal of manpower, funding, and expertise. Yet, all too often, efforts to develop aquaculture industries fail due to market conditions, not lack of production technology expertise. There is a significant need to attempt to develop some simplified and rapid market assessment techniques that are applicable to the Latin American context.

Polling firms in the U.S. have identified indicator questions and variables that can be used as predictors of behavior (Burns and Bush, 1998). These are based on analysis of comprehensive survey data and statistical analyses that develop relationships among different variables and parameters. For new industries like aquaculture in developing nations without the plethora of databases, surveys, and census information that some other countries have available, these types of convenient shortcuts are not possible. The surveys conducted in Work Plan 9, however, provide a wealth of information related to fish and seafood markets in Central America. If a reliable subset of key parameters can be developed, it may be possible to develop a simplified and relatively rapid market assessment tool. The tool would need to be evaluated carefully to define its usefulness to other regions of Latin America and with species other than tilapia. If successful, the potential impact and value of this tool would be quite high.

Rapid assessment tools have been developed for other types of development initiatives. For aquaculture ICLARM is actively using a spreadsheet-based resource allocation interactive tool to assist with community and farm development (Prein et al., 1996). This tool has successfully been used to identify sustainable approaches to resource use and economic development. Hatch and Falck (2001) are working to develop a rapid assessment tool to assess economic risk in tilapia production in Honduras. A spreadsheet program is used that allows for distributions of costs, yields, and prices, to be entered to assess the effects of economic risk in production. However, there is no indication in the literature of the development of such a rapid assessment methodology for addressing marketing feasibility.

Quantified Anticipated Benefits

Direct beneficiaries of this project will be aquaculture growers in Peru and Mexico where the methodology developed will be tested. The testing efforts will provide valuable market information on markets for tilapia and for other important native species.

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* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

The Mexican student who will use this project as a basis for an MS degree in Mexico will benefit from this effort as well as provide for human capital development in marketing analytical techniques.

This methodology has potential to be a valuable tool for many areas where aquaculture is a new technology, or where a new species or product is to be introduced.

Research Design

Location of Work: The methodology will be developed at the University of Arkansas at Pine Bluff using survey data obtained in the Ninth Work Plan. The methodology will then be tested in both Mexico and in Peru.

Methods: The econometric and statistical analyses to be conducted in the discrete choice models previously described will identify statistically significant variables related to market characteristics for farm-raised tilapia. These significant variables will be compiled into a short survey instrument. Other, non-significant variables will be selected at random under each major heading on the survey (sales, supply, attitudes, socio-demographic characteristics, and economic characteristics) for inclusion in the short survey instrument. These non-significant, randomly selected variables, will provide a basis for considering errors of omission in the rapid assessment methodology.

The study on marketing costs (LP analysis) will provide insight into those costs and factors that present the greatest risk to tilapia farmers and those that are the most important determinants of market feasibility. These cost factors will be condensed into a second short survey instrument.

The rapid market assessment tool will be composed of these three components: market characteristics (including price points), marketing costs and margins, and production costs. The decision criterion for determination of feasibility is whether market price minus marketing cost minus production cost is greater than zero. If it is, then the market segment in question is deemed to be feasible. If the result is less than zero, then targeting that particular market segment is deemed infeasible.

Testing of the market assessment tool will be carried out in Mexico and in Peru. In Mexico, the principal market area and population center is Mexico City. Both tilapia and native cichlid species are sold in Mexico City. Native cichlid species are highly desired products by Mexican consumers and have potential as native species for development of culture techniques. In Peru, Lima is the major population center, and will be used as a testing site. Both tilapia and *Colossoma* spp. are sold there. However, Peru offers the interesting alternatives of Iquitos, a significant population center in the Amazon region where tilapia is banned, but *Colossoma* spp. are sold along with other freshwater species. Tarapoto further offers a study area in an aquaculture production area characterized by low income, small-scale aquatic farms. In each study site, a random sample of restaurants and supermarkets will be drawn from telephone listings. In open-air fish markets, vendors will be selected randomly using systematic spatial sampling techniques (Burns and Bush, 1998).

Direct personal interviews will be conducted using the short survey instruments developed. Statistical comparisons will be made between the responses for variables that were significant in the Central American surveys and the responses to questions related to variables that were not significant. This comparison will provide an indication of whether the selection of variables for the short survey omitted some key factors.

Data will be processed to determine price points for product sizes and forms along with projected sales volumes as determined by the first short survey for various market outlets. Data from the second short survey will be synthesized into an estimate of marketing costs for various product sizes and forms. Production cost estimates will be developed from enterprise budgets. Production and marketing costs will be subtracted from the relevant price points for various product sizes and forms for the different market channels and segments. Positive values will indicate feasible markets.

Regional Integration

The Regional Plan for South America refers specifically to the identification of marketing strategies to optimize economic returns. Research needs reiterate the importance of determining optimal marketing strategies for *Colossoma* spp.

Schedule

7/1/01	Initiate project.
12/31/01	Rapid market assessment methodology developed.
7/31/02	Complete testing in Mexico City.
12/31/02	Complete testing in Peru.
4/30/03	Complete final evaluation of effectiveness of rapid market assessment methodology and submit final report.

Literature Cited

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Hatch, L.U. and J. Falck, 2001. Assessing economic risk in tilapia production in Honduras. Abstract. Book of Abstracts. Aquaculture 2001, World Aquaculture Society, Orlando, Florida.

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Regional Enterprise Budget and Business Plan Development

Marketing and Economic Analysis Research 3 (10MEAR3)/Activity/Kenya

Collaborating Institutions
Moi University, Kenya
Mucai Muchiri

University of Arkansas at Pine Bluff
Carole R. Engle

Objectives

1) Collect and organize the information necessary to prepare enterprise budgets and business plans for several fish production systems.
2) Promote business-oriented thinking among farmers and encourage record-keeping much needed for effective extension efforts.

Significance

There is a great deal of interest in the development of successful aquaculture businesses in the East Africa region. With the development of the new core site in Kenya, there is an important opportunity to utilize economic planning tools as a basis for an integrated research-outreach effort designed to have a quick and substantial impact on the region. Recently, a workshop organized in Sagana, Kenya, identified and emphasized the need for business planning as critical to the success of the aquaculture sector in the country. This activity will identify the gaps in information that are critical to the development of comprehensive business plans for aquaculture businesses. PD/A CRSP field research studies could then be designed to fill in the gaps in production information to fully support the incipient aquaculture businesses in Kenya and the East Africa region.

Quantified Anticipated Benefits

The proposed activity will produce enterprise budgets and pro forma financial statements for business plans that can be used as guides for prospective and existing fish farmers. A sound business plan is required by financial institutions to obtain credit. The information developed under this work plan will not only provide farmers with the appropriate tools to show profitability, but also help lending institutions to better assess the viability of aquaculture projects and reduce the rate of failure in loan repayment. Furthermore, this effort will identify the gaps in the existing database on fish farming in Kenya and the region. These gaps can then be addressed through subsequent CRSP-funded research. This will reduce the time for the overall project to demonstrate an impact in the region.

Activity Plan

Location: The majority of the analytical work will be conducted at the University of Arkansas at Pine Bluff (UAPB). However, there will be close collaboration with the PD/A CRSP Africa project and Moi University. Several trips to Kenya over the course of the activity will be made to collect additional information as needed and for the training component implemented as part of this activity.

Methods: The Regional Plan for East Africa identifies "Regional Enterprise Budget Development" as a key activity for the new Africa site. The effort to develop enterprise budgets and pro forma financial statements for business plans has been initiated. A workshop was organized at the Sagana Fish Farm, Sagana, Kenya, September 17-19 1997 with the main objective being to provide joint input into the ninth work plan. Additional objectives were to 1) identify local constraints to the development of aquaculture, 2) define the current state of knowledge about selected production systems, 3) establish the mechanism of data collection and analysis, 4) regionalize aquaculture

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

objectives and 5) recommend actions to be taken to attain local and regional objectives. Recommendations of this workshop emphasized the need for aquaculture business planning along with a reinforcement of the linkages between research and extension. Following the recommendations of the workshop and in accordance with the Regional Plan for Africa, this activity will lead to the collection of production and economic data relevant to selected production systems. A standard form will be designed for data collection. Data collected will enable the development of enterprise budgets necessary for business planning. During this effort, the information gaps will be addressed.

Enterprise budgets will be developed for the production systems selected. Production systems will utilize tilapia as the primary species in either monoculture or polyculture with *Clarias spp.* Two principal levels of production systems will be analyzed: 1) non-cash systems based on on-farm inputs; and 2) commercial systems based on purchased feeds and fertilizers. Standard conventions and procedures as presented in Kay (1994) will be used to develop the budgets. However, the presentation of the budgets will follow both the U.S. and the host country's methods of budgeting. For those cost and return items for which data are lacking or deemed insufficient, sensitivity analyses will be conducted to present a range of potential economic outcomes from a particular enterprise. These same items will be those identified as constituting gaps in existing knowledge and for which additional research may be recommended. The enterprise budgets developed will be published for distribution with instructions to farmers on how these can be adapted to their particular situation and farm.

From the enterprise budgets, pro forma financial statements (including annual cost and returns, estimate of total required capital, balance sheets, income statements, and cash flow budgets) will be developed for the production systems and average farm sizes selected. These statements will be in a form usable for presentation in an aquaculture business plan or loan proposal. Any additional gaps in the knowledge base identified in this activity will further be specified as those needing additional research. The financial statements will be published with instructions containing a spreadsheet for farmers to enter farm-specific data as needed.

A summary of marketing information will be prepared for each of the production systems/scenarios proposed. Fundamental market information required to present the market plan component will be included. This will include information on prices, product forms, market channels, volume requirements for various market channels, potential target market segments, etc.

A workshop will be organized and held in Kenya by the PD/A CRSP representative and the project Co-PIs. The workshop will be used to present and train participants to interpret the enterprise budgets, financial statements and marketing information compiled. While there is a wide range of educational levels among Kenyan farmers, the general educational level is higher than that commonly assumed in developing countries. The general level of record-keeping is likewise higher than commonly assumed. It will further present methods for using this information to acquire financing for aquaculture businesses and to use this planning base for decision-making on the farm.

Regional Integration

This activity responds directly to the first objective listed in the Regional Plan for East Africa. Although the 1997 Sagana workshop did not include representatives from other African countries, recommendations of the workshop addressed regional objectives and concerns and included tentative plans for future collaboration in Tanzania and Uganda. This effort to develop enterprise budgets and financial statements for aquaculture will ensure that production systems selected will be appropriate and relevant to Kenya. The marketing information will be from the potential markets accessible to the proposed production units and will be compiled by major potential target markets.

Schedule

7/1/01 Initiate project.

6/30/02 Enterprise budgets will be compiled along with the necessary sensitivity analyses for the production systems selected.

4/30/03 Pro forma financial statements will be completed. Marketing information reports will be completed and made available. A workshop will be organized. Final report will be submitted.

Literature Cited

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Economic and Risk Analysis of Tilapia Production in Kenya

Marketing and Economics Analysis Research 4 (10MEAR4)/Study/Kenya

Collaborating Institutions
Moi University, Kenya
Mucai Muchiri

University of Arkansas at Pine Bluff
Carole R. Engle

Objectives

1) Estimate the expected net returns, breakeven cost, and breakeven yield of tilapia farming in Kenya.
2) Estimate the likelihood of achieving profit (positive net returns) and the distribution of outcomes for total revenue, total costs, breakeven yield and breakeven price for varying farm scenarios.

Significance

Further growth and development of the tilapia industry in Kenya will depend upon its profitability. Estimates of net returns are essential for both the prospective producer and the lender to understand whether or not the proposed enterprise is expected to be profitable. Moreover, the level of profitability is important for comparison with other possible alternative enterprises. Enterprise budgets can be used to identify the overall expected level of returns and the level of costs that will be required. Enterprise budgets provide a means for analyzing a potential enterprise before resources are committed to it.

Enterprise budgets are based on single, usually mean, values for prices, costs, and quantity values included in the budget. However, market, financial, and production risks result in fluctuating prices, costs, and yields. An individual who is making management decisions on a fish farm will be faced with a variety of risks. Information on the varying likelihoods or probabilities of losses that would result from different management options would help farmers and planners make better decisions. Those individuals who are extremely risk averse would select those management options that are less likely to result in losses whereas more risk-tolerant individuals might opt for riskier options that have a chance of producing high levels of profits. In either case, information on the riskiness of management options would provide guidance for decision makers.

Quantified Anticipated Benefits

The primary direct beneficiaries of this study will be tilapia growers and agricultural lenders in Kenya. Enterprise budgets form the foundation of a business plan. Detailed enterprise budgets that address a variety of farm situations will provide a broad overview of profitability of tilapia production under a variety of different scenarios. The sensitivity analyses will further shed light on how robust are the estimates of net returns and what factors are most likely to affect the profitability of tilapia production. The risk analysis will provide further evidence of the confidence with which growers and lenders will be able to have in this type of production enterprise.

Secondary beneficiaries will include the university and extension personnel. All aquaculture programs rely upon enterprise budgets as the basis for discussions of the profitability of aquaculture enterprises. These budgets would be expected to be used in the classroom and by extension personnel as they work with prospective new tilapia growers.

The Moi University student will benefit from this project by using it as the basis for his/her thesis project. The student will learn the basic skills needed to develop enterprise budget and risk analyses.

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* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

Kenya and the region will benefit from the availability of fundamental enterprise budgets as well as the increased human capital that will result from this project. The region will also benefit from the economic growth anticipated from enhanced market development and profitability of tilapia farms. The PD/A CRSP will be a secondary beneficiary because a more complete foundation of economic information on the profitability and risks of tilapia production in Kenya will assist lending institutions to make better decisions on loan applications. This should result in improved success rates of aquaculture loans and this should result in growth of the tilapia industry in Kenya.

Research Design

Location of Work: The majority of this work will be done at Moi University in Kenya. There will be close communication with the cooperators at UAPB.

Methods: Economic engineering techniques will be used to develop a set of enterprise budgets for tilapia production in Kenya. Different scales of production will be defined based on the ranges of sizes of tilapia farms in Kenya. Separate budgets will be developed based on each scale of production. Typical yields and fish prices will be used to calculate gross revenue. Typical quantities of fixed and variable resources used in the production of tilapia will be specified. The budgeted values will be used to calculate net returns to operator's labor, management, and risk. Breakeven prices and costs above variable and total costs will be calculated.

The profitability of tilapia farming under conditions of risk and uncertainty will also be evaluated. The risk analysis will be conducted as a stochastic simulation using Crystal Ball™ software, a spreadsheet add-in program that allows the incorporation of uncertainty in risk analysis models (Decisioneering, 1996). This program has been used previously for bio-economic modeling of aquaculture firms (Zucker and Anderson, 1999) and to analyze the profitability of shrimp farming in Honduras under conditions of risk and uncertainty (Valderrama and Engle, 2001). In the simulations, ranges of values that random variables such as yield and prices may take are defined by probability distributions instead of the sample averages used in standard enterprise budgets. Monte Carlo simulation techniques (500 iterations per simulation) will be used to generate values for individual cost and quantity items based on the probability distributions. Results presented will include the entire range of possible outcomes for parameters such as gross receipts, total costs, and net returns, as well as their associated probability.

Normal distributions will be used to define tilapia yield and price. These parameters are highly variable and influenced by many factors. Yield is determined by stocking densities, feeding rates, cycle length, and overall survival, but is also influenced by weather patterns that fluctuate randomly. Farm prices depend on marketing strategies and supply-demand interactions. As with many biological processes, these uncertain variables can be described best by a normal distribution (Zar, 1999).

Production costs will be described by triangular distributions based on the most likely value (means included in the enterprise budgets) and minimum and maximum values determined from the original data for each scenario. Triangular distributions are considered to provide the best representation of estimates when only a small number of data can be obtained (Taha, 1988).

The likelihood of achieving profit (positive net returns) and the distribution of outcomes for total revenue, total costs, breakeven yield, and breakeven price were calculated for each farm scenario. Overlay and bar charts will be developed to compare the distribution of outcomes among farm scenarios and to draw inferences from the risk analysis.

Regional Integration

The Regional Plan for Africa refers specifically to planning and implementing economics and marketing research activities in the region. Research needs for tilapia culture specifically refer to economic analysis and market development.

Schedule

10/1/01	Initiate project. Conduct initial planning meetings with project participants. Select the student.
11-12/01	Collect secondary information on prices of tilapia, costs of inputs, and farm yields of tilapia.
3/30/01	Have prototype spreadsheet model developed and ready for validation.
6/30/02	Have enterprise budget models completed.
9/30/02	Complete collection of primary data necessary from tilapia farms.
12/30/02	Finalize enterprise budgets.
3/30/02	Complete risk analyses.
4/30/02	Submit final report and have a manuscript prepared from this project.

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Institutionalizing Techniques for Building Hillside and Levee Ponds for Water Supply and Aquacultural Development in Latin America

Adoption/Diffusion Research 1 (10ADR1)/Study/El Salvador, Honduras, and Nicaragua

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Objectives
The overall goal of this study is to collect and develop information required to institutionalize pond design in Honduras and Latin. This study is coordinated with the training activity outlined in other Honduras Project investigations.

Specific study objectives are to:

1) Estimate local runoff coefficients (NRCS curve number parameters, rational runoff coefficients and soil seepage parameters for an existing Excel model developed for the design of hillside/ watershed catchment ponds for water supply development; and,
2) Develop strategies for designing and managing sustainable levee ponds for aquacultural production that reflects local conditions based on local water supply and soil suitability for pond development.

Significance
The hillsides of Latin America cover about 1 million km^2 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 Latin 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), 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). Fishponds also serve multiple roles including water conservation, income generation and food production. However, hillside ponds are rare in Latin America apparently because of high costs associated with mechanized earth moving and/or high labor needs for hand construction, and lack of knowledge of alternate designs suited to local conditions. Further, research by both Zamorano (Lee, 1997) and CIAT (1997) suggests that poor understanding of biophysical (landscape) and socioeconomic (lifescape) linkages among farmers in hillside watersheds impedes more sustainable use of land/water resources in Honduras, and by extension, to neighboring countries in Latin America.

Previous objectives (Verma et al., 2001) were to 1) elicit farmers and change agents perspectives about the role of aquaculture within hillside watersheds; 2) identify design criteria for hillside ponds in participation with end users; and 3) develop alternative pond designs suited to local conditions. The team achieved excellent progress toward identifying NGO and other change agent groups within Honduras. We intend to continue this process in surrounding countries via the website and on-site visits via scheduled training sessions.

The process of design criteria identification was systematized by developing computer models (Verma et al., 2000). As design criteria were identified, it became apparent that the water supply function should be separated from the aquaculture production function, enabling production pond design and development in regions where springs and other sources provided adequate water. In regions where water supply was to be developed by capturing precipitation runoff, a watershed/hillside pond design model coupled with the aforementioned aquaculture production model will be used in tandem. Six regions had good quality monthly precipitation data. One region had reasonably good precipitation-depth-duration data. Local intelligence is required to estimate how one can extrapolate these data to the six regions. The hillside/watershed catchment model evaluates a monthly water balance and determines a minimum watershed size for a desired pond size, nine out of ten years. The minimum watershed size depends on the watershed steepness and cover. Since the actual watershed size may be larger, principle and emergency spillways are designed to handle the 2-year and 10-year storms, respectively. The levee pond model was constructed to perform a monthly water balance, where evaporation, seepage, direct rainfall and incoming pumpage/springflow rates are the major components. Average volume change is the management output along with time to fill. The levee pond by definition receives no runoff from surrounding lands.

The original approach for objective 3 was to develop designs for each component of a pond and custom design a pond for various representative micro-watershed by integrating appropriate component as is in modular design. It became apparent that this modular design approach was not suitable for the range of variability found in Honduras and the region. Thus, the strategy was shifted from modular design approach to the development and application of computer models by knowledgeable practitioners. The catchment (hillside/watershed) and production (levee) models were developed on the Excel platform due to widespread presence of this platform among NGO groups. The English version of the models is essentially complete, although a detailed users guide is yet to be developed. A Spanish version will be produced. Adding a local soils expert to the team will greatly augment the design effort in that key input parameters will be refined and procedures for assessing the parameters will be institutionalized at the local level.

Aquacultural production need not be the sole purpose of waters contained in the hillside/watershed pond. We need to communicate these results to contractors and engineers involved in water supply development and aquacultural production. Additional local intelligence is needed to refine input parameters related to runoff coefficient estimation, seepage rates associated with various soil types and monthly evaporation rates at various regions within the country.

In this study, tools that have been developed by UGA, Zamorano, CIAT, and the SANREM CRSP (Bellows et al., 1995) will be used in a participatory setting with NGO groups to elicit their perspectives on linkages between pond aquaculture operations and watersheds in two countries surrounding Honduras. This effort will build upon the recent experience in Honduras (Molnar et al., 2001; Verma et al., 2000). We believe that this will lead to an increased understanding (among farmer groups and technical assistance personnel) about how natural resources within watersheds can be utilized in a more sustainable fashion. Additionally, we will use concurrent (implying a participatory process) engineering design principles (Veland, 1992) to identify the needs of fish farmers ("customers") interested in hillside aquaculture, their socioeconomic and environmental constraints, and the level of technologies available to construct ponds. These criteria will then be used to develop alternate designs (which address farmer needs and constraints) that can be used for pond construction following the Honduran strategy presented by Verma et al. (2000).

The timing for water development work in Latin America is excellent given the in-country recovery effort following hurricane Mitch. Considerable data collection efforts relating to soils and runoff estimation are currently underway. Thus the time is right for dovetailing modeling parameter needs with other ongoing efforts. The models provide a platform for comparing and contrasting water runoff and sediment transport from watersheds under various cover conditions.

Quantified Anticipated Benefits
This work will result in a hillside/watershed Excel-based model and an aquacultural production pond model. Seepage rate parameters, curve number parameters, permeation rate and peak runoff parameter data for important fish production regions in Honduras will be determined. Four to five training sessions for NGOs, engineers and contractors, with 25 persons per session, will be completed. Two or three students from Zamorano will be partially reimbursed for senior level tuition expenses. Instrumentation for measuring location (GPS), soil texture, seepage and hydraulic conductivity will remain for continued use at Zamorano.

An improved understanding of biophysical and socioeconomic linkages between aquaculture and the associated watersheds has important 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 will 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 Zamorano staff will gain. The lack of such interdisciplinary training has been identified as a major weakness of the National Agricultural Research System (NARS) in Honduras (Contreras, 1992).

Research Design
Location of Work: The field work for this study will occur in a representative hillside micro-watershed in the Comayagua department of Honduras. A major portion of the work will, however, will occur at Zamorano and at UGA campuses where additional facilities and expertise is available.

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

Identification of Representative Micro-Watersheds and Parameter Determination
The first task is to identify one or more micro-watersheds that typify hillside/watershed pond regions and has existing fish farmers, including operators who use land and water resources for a range of agriculture-related activities. A team of UGA and Zamorano personnel will do watershed identification and organize the field testing expeditions. Soil samples collected near the surface and at 1 ft intervals at the selected locations will provide soils information. Seepage pits are scheduled for seepage evaluations. Permeability data taken using Guelph permeameters will provide additional seepage information. Curve number parameters and runoff coefficient data will be visually determined. If time permits and a suitable location is available on the Zamorano campus, a demonstration pond and water measurement facility is scheduled. Permeameters and other sampling apparatus are scheduled to remain at Zamorano.

Institutionalizing the Design Process
The working team for the pond design work will come from UGA, Auburn and Zamorano PI's, students from Zamorano, interested farmers, extension agents/NGOs, and local leaders. A fundamental objective of this project is to convey the models and user guides to these groups via the website. Training programs are the vehicle to seed the process. Posting the Excel models on the RDS website combined with training sessions for local engineers and contractors is the primary delivery approach. A demonstration site is scheduled for Zamorano. The pond designs developed are scheduled to be documented and discussed with farmers during a follow-up trip to Honduras (with the same working team). The Excel models and the users guides are scheduled for Spanish translation.

Regional Integration
We anticipate expanding the activities of this project to surrounding countries in Latin America by including travels to identify potential NGOs with interest in aquacultural production. Focus group

sessions and training sessions (see the work plan for 10ATR1) are scheduled to be completed in Nicaragua and El Salvador, in addition to Honduras. Model delivery and training sessions are likewise scheduled in these countries. This work will be coordinated with efforts of the RDS website development work.

Schedule

Work will commence by September 2001. Initial tasks (identification of watersheds, strengthening of institutional linkages, and refinement of design parameters) are scheduled for completion by December 2001. Soil sampling expeditions are scheduled for completion for completion by December 2001. The Focus group tasks (Honduras, Nicaragua, El Salvador) are scheduled for completion by March 2002. Final training and model delivery (Honduras, Nicaragua, El Salvador) is scheduled for completion by March 2003.

Report Submission

Two reports, spanning the two years of this study, are scheduled for submission to the PD/A CRSP by 30 April 2002 and 30 April 2003. Final report to be submitted by 30 April 2003.

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Income, Food Security, and Poverty Reduction: Case Studies of Functioning Clusters of Successful Small-Scale Aquaculture Producers

Food Security Research 1 (10FSR1)/Study/Honduras

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Objectives

1) Identify clusters of small- and medium-scale producers that have engaged in repeated cycles of tilapia production in Santa Barbara and El Paraiso, Honduras.
2) Elucidate the circumstances and conditions that contribute to successful implementation and continued practice of tilapia culture.
3) Formulate principles and guidelines for providing technical assistance and research support for small- and medium scale tilapia farmers in Honduras.

Aquaculture plays an identifiable role in helping rural Hondurans achieve food and income security[1], but there is a need for better understanding of how aquaculture works at the village level (Molnar and Lovshin, 1995). Lessons learned from actual circumstances where tilapia culture is a regularized component of local farming systems could provide realistic guidance for the network of national and regional institutions dedicated to advancing aquacultural development. Another constituency for this information lays in the broader aggregate of agencies and organizations that feature aquaculture as one component in their array of development interventions. Understandings gained from case studies of successful clusters of practicing fish farmers can contribute to the goal of better directing aquaculture's inclusion in current and future integrated community development initiatives.

Significance

Small-scale aquaculture in Central America has often failed since efforts began over more than two decades ago (Lovshin et al., 2000). Enough abandoned ponds exist that one might be tempted to question whether aquaculture has proven to be an inappropriate tool for reaching development goals in Honduras. However, some small farmers have experienced remarkable success, and their ponds have provided real food security and income benefits for the families involved. This study will identify how clusters of practicing tilapia small farmers manage and sustain their fishponds and describe the experiences, circumstances, and resources that enable their successful realization of the enterprise.

Implementing small and medium scale aquaculture on a widespread and sustained basis is a long-term process (Molnar et al., 1991; Harrison, 1993). In Honduras, tilapia development has taken place during

[1] Vitamin A deficiency) and iron deficiency anemia have been recognized as public health problems in Honduras for over 30 years. The dietary Fe status of the population in rural west of Honduras was found to be very poor. Extremely low intakes of fruits and vegetables and of meats, coupled with significant intake of coffee by all age groups, further limits the availability of dietary Fe in the Honduran population. (Ohri-Vachaspati and Swindale, 1999). Tilapia culture could ameliorate dietary inadequacies in communities containing clusters of producers that ensure availability of fish to women and children, but Bergeron and Banegas (1998) suggest that cross-sectional, group interviews are not reliable means for assessing levels of, or changes in, food security.

an extended period of administrative reorganization and political uncertainty that is directly related to the low quality of government services that are available to the rural sector (Mendez, 1986). The tilapia industry is still recovering its growth pattern after hurricane Mitch, but has more recently had to deal with fingerling shortages and disease problems. Many regard progress as inadequate and no large-scale donor-support for technical assistance is likely to emerge. Thus, it is vital that this project fortifies Zamorano's ability to sustain aquaculture development in Honduras because other sources of technical assistance have diminished and farmers must learn to utilize private firms and nongovernmental organizations for services and technical assistance. [2]

Despite setbacks and disappointments, some small- and medium scale producers have incorporated tilapia culture into their farming systems. Knowledge of actual "secrets to success" may lead the many public and private nonprofit agencies endeavoring work with Honduras communities to better understand the proper role of aquaculture in Central American rural development. These insights may help donors and practitioners to incorporate aquaculture effectively into current and future development programs. Many nongovernmental organizations have resources and personnel trained in tilapia farming and fish farming already is a part of the portfolio of activities which local advisors are prepared to provide technical advice. The findings may facilitate realistic consideration of the prospects for promoting fish culture as a farm enterprise in some locales and discouraging investments in pond construction and land diversion in other. The results of the case studies will identify the configurations of circumstances that enhance the viability of the enterprise where it presently is an established and productive activity for clusters of successful farmers[3].

There may be elements of mutual support and collaboration that enable groups of farmers to be more successful collectively than they would otherwise be individually. Neighbors may provide information and problem solving to one another in the absence of adept technical assistance from public and private sources. What extension assistance is available may resonate among a cluster of farmers more effectively than if delivered to an individual producer, as such information is often subject to local interpretation and refinement before it can actually solve a farmer's problem or advance the production process.

Rural communities in Honduras have experienced little support and many inadequacies in the performance of their government (Stonich, 1991, 1992). With USAID encouragement, Honduras undertook a land-titling project in 1982 with the expectation of increased access to credit for small farmers, and subsequently, increased on-farm investment and enhanced tenure security. Larson et al. (1999) suggest that titling by itself did not bring the intended outcomes, and that institutional factors had more immediate effects on the ability of small farmers to improve their farming systems. Factors found to have a significantly positive effect on technical efficiency, directly or indirectly,

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[2] Honduran rural women play an important role in agriculture, especially in the peasant and small farmer sectors, working an average of four hours a day in crop and livestock activities. Women are responsible for establishing a survival strategy for the household unit. Women, who bear the entire responsibility for agricultural production, head about 20% of rural households. Development policies, however, still consider men as producers and women as responsible only for household tasks. Women are primarily responsible for vegetable gardening and small livestock - poultry, pigs and goats. While little data is available on the gender division of labor in agriculture, women take part in most activities and predominate in food processing. In fisheries, women also work primarily in processing activities. Women are responsible for household tasks, including water supply. Men contribute significantly to fuel wood collection. In households in which women are active in agricultural production, men and women often share the decision-making. Men predominate in decisions in regard to type of crops, varieties, and fertilizers, while women generally have a greater say in family expenses and pricing of produce (Fleck, 1996). <www.fao.org/sd/wpdirect/WPan0005.htm>

[3] Based on a survey of 48 communities in central Honduras, Pender et al. (1999) identified six major pathways of development in central Honduras since the mid-1970s: basic grains expansion communities; basic grains stagnation communities; coffee expansion communities; horticultural expansion communities; forestry specialization communities; non-farm employment communities. Tilapia culture is most likely to complement farming systems in coffee and horticulture-based communities. The pathways were distinguished by factors determining comparative advantage, including agricultural potential, population density, and access to markets and technology. Pender et al. (1999) maintain that the key causes of change in productivity and resource management are different and more pathway-dependent than the key causes of change in poverty, which depends to a great extent on provision of public services. Basic infrastructure and public services are badly needed throughout most of central Honduras, while efforts to address sustainable agricultural development may not be sufficient to solve poverty problems. There may not be large tradeoffs between achieving more sustainable development and reducing poverty, since the causes are different.

were education, technical assistance and credit. The dummy variable for land titling was consistently non-significant.

Fingerling production is critical concern for small and medium scale fish farmers seeking to restock ponds and begin another cycle of production. Some communities may feature a lead producer who is particularly adept at managing broodstock and organizing the reproduction process. Such lead producers may support their neighbors by ensuring the availability of quality seedstock at predictable intervals that enables repeated cycles of production. Case studies of successful clusters of producers may elucidate the ways these core producers induce other farmers to build and stock ponds with tilapia. Results of past studies show that human capital (e.g., schooling, literacy), wealth, and security of land tenure help farmers adopt new farm technologies. These studies have focused on villages with tight links to the market and little land. Gorday et al. (2000) considered whether these patterns also apply to more self-sufficient economies with ample land. Their analysis of 101 Tawahka Indians households in Honduras's rain forest suggests that education and knowledge of Spanish enhance adoption by facilitating the flow of information into the household and by making it easier for people to judge the quality of the technology.

Certain agglomeration advantages may accrue to clusters of producers who are better able to respond to market demand for fish and facilitate connections to buyers seeking fish for resale, restaurant use, or home consumption. "Coyotes" or middlemen who supply tilapia to urban markets may find it more useful to call on known clusters of producers with greater likelihood of finding available quantities of desired sizes of fish. Again, some lead producers in a community may have connections to processors, resellers, or others in distant markets that increase the reliability of marketing harvests at anticipated prices. Thus, clusters of producers may experience certain advantages in production, restocking, and the outlined program of case studies can clarify the role of marketing in sustaining the practice of fish culture.

Ruben and van den Berg (2000) examined wage income as a major element of the rural livelihood strategy that permits the maintenance of a survival strategy based on the combination of a number of complementary activities. This enables small farmers better to overcome limitations in the access to markets and favors the adoption of risk-sharing strategies that are considered typical for resource-poor households operating under conditions of selective market failure. Their work in Honduras suggests that one segment of small farmers may view fish culture less as a livelihood and more as a lifeline, i.e., a source of diversification and risk reduction.

Kurbis (1997) identified several conditions contributing to successful fish farming in Honduras. The US Peace Corps, through which plans were made to effectively utilize the research results, ceased their aquaculture efforts after 1995 budget cuts. Even more confounding, other extension agencies involved in aquaculture were found to have neither the organization nor the resources to effectively promote aquaculture. This proved difficult to overcome because biological research results typically outline the biological, and sometimes economic, circumstances under which sustainable success is likely. It is also often assumed that extension already understands the basics of the technology. Detailed prescriptions for fish culture often are less than useful to extensionists whose expertise lies in other areas and who have had only piecemeal training in aquaculture.

Buckles (1999) found that the relative profitability of velvet bean (mucuna) in Honduras was enhanced by seasonally high maize prices during the second season when maize is harvested. Relatively easy access to land through inexpensive land ownership and land rental markets has made it possible for even small-scale farmers to dedicate land to this farming system. In a parallel fashion, farmer perceptions of tilapia culture as viable livelihood may be the primary factor in farmers' decision making about the adoption of fish as a component of their farming systems. Case studies may reveal the material circumstances that lead to perceptions of viability, as well as the organizational and contextual factors that support the practice of fish culture.

Institutional inadequacies in the kind of research and extension support provided to the aquaculture sector in Honduras cannot be ignored. Kurbis (1997) found it very frustrating to have gone to great lengths to complete research only to find that it could not be used effectively because insufficient government resources were devoted to aquaculture. He concluded that future initiatives that combine competent extension with effective targeting could help tilapia culture to realize its potential for improved food and income security in rural Honduras. It is not realistic to expect a dramatic change in the quality of assistance available from the public sector in Honduras. Nonetheless, the capability of nongovernmental organizations providing technical assistance in aquaculture is improving, in part due to the previous two years of effort by a PD/A CRSP project (Verma et al., 1999). Loker (2000) reviews the problems associated with a newly implemented development project in Santa Barbara, exploring explores the social causes of project failure.

Quantified Anticipated Benefits

Aquacultural development may generate both direct and indirect benefits. Benefits accrue directly to participants by increasing their access to food, income, and nutritional assets, by increasing their human capital (through training, better health status, finishing more years in school, etc.), and by increasing both household and community social capital (increasing community cohesion, increasing community propensity to act jointly for the benefit of members of the community, trust and empowerment). It may also provide such benefits as decreasing the time spent on less productive farm activities, decrease the cost to households of obtaining protein, among others. It is important to note that a single fish culture intervention can have many of these positive effects. For instance, a pond rehabilitation and expansion investment can improve the capacity and physical environment of a community, can increase food availability, income, and nutritional security. Aquacultural development can increase social capital through the skills, networks and confidence gained by the producers' interactions with other producers and input suppliers associated with the implementation of their fish culture enterprise. There are also indirect benefits, such as more positive and beneficial interactions between producers and their communities.

Research Design

To address these issues, the study will conduct case studies in communities that have experience with aquacultural development. Based on reviews of available documents, interviews with officials, extended conversations with fish farmers, and other sources of information cases studies will be developed. The objective is to conduct an in-depth analysis of the impact of aquacultural development in selected communities in two Honduras departments—El Paraiso and Santa Barbara. Because aquacultural development may operate in different ways in different regions, an attempt has been made to choose locations that are geographically dispersed and represent diversity in rainfall and elevation.

Both locales have known clusters of successful tilapia producers; yet represent contrasting physical and social environments reflecting the diversity of social and physical conditions in Honduras. A cattle and basic grain-growing area, El Paraiso represents the lower elevations, broad valleys and low mountains of the Choluteca river and its tributaries. A coffee-producing region, Santa Barbara represents conditions of higher elevations, sharper valleys, and more evenly distributed rainfall. A higher proportion of the population of Santa Barbara is descended from indigenous peoples.

The study will produce a foundation report that presents detailed case study findings, their implications for NGO and governmental efforts to provide technical assistance. In additional, a Spanish-language leaflet will be produced that summarizes, illustrates, and highlights the study findings. This publication will be widely distributed in Honduras and Central America as a means for providing baseline principles for initiating and guiding technical assistance in aquacultural development. Both publications will be made available on the web site established during the previous project period.

Regional Integration

The proposed research will integrate with regional plans in a number of fundamental ways. It will seek to implement its work in consultation with CIAT and PRADEPESCA, the regional FAO-sponsored entity for fisheries and aquaculture. The study results will be made available to NGOs and government agencies in Central America that are positioned to implement its recommendations for reaching, stimulating, and supporting small- and medium-scale producers in the region. The results will be used to guide the other components of this project in terms of providing its key insights to NGOs and others that are considering augmenting or adding their capability to provide technical assistance in tilapia culture.

Schedule

Preparation (background, selection of case study communities): July 2001 – November 2002
Data collection and analysis: September 2001 – May 2002
Analysis and writing: June 2002 – January 2003
Completion of case studies: January 2003
Completion of synthesis report (printed leaflets): March 2003
Dissemination of synthesis report and printed leaflets: April 2003
Final report to be submitted no later than April 30, 2003.

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Characteristics of Fish Buyers Likely to Purchase Farm-Raised Tilapia in Honduras and Nicaragua

Product Diversification Research 1 (10PDVR1)/Study/Honduras and Nicaragua

Collaborating Institutions
Escuela Agrícola Panamericana, Zamorano, Honduras
Freddy Arias
Dan Meyer

Universidad Centroamericana, Nicaragua
Agnes Saborio

University of Arkansas at Pine Bluff
Carole R. Engle

Objectives

1) Identify those characteristics and preferences of restaurants, supermarkets and open-air fish market vendors in Honduras and in Nicaragua that sell tilapia, those that used to sell tilapia, and those that never sold tilapia.
2) Identify those characteristics and preferences of restaurants, supermarkets and open-air fish market vendors in Honduras and in Nicaragua that are likely to add tilapia to their product lines.

Significance

Tilapia growers in Honduras and in Nicaragua have requested guidance identifying the most promising markets and in developing efficient marketing strategies. Small and medium-scale producers, in particular, do not have adequate resources to conduct market research and fewer resources to conduct advertising campaigns and to develop markets. Identification of very specific types of market outlets that are most likely to either sell more tilapia or to begin to sell tilapia would enable tilapia growers to minimize their marketing expenses by targeting those types of markets likeliest to purchase their products.

Discrete choice analysis using qualitative dependent variables has been shown to be an effective analytical tool in the analysis of perceptions and attitudes. While several studies have used these techniques in U.S. markets for aquaculture products, they have not been applied to Latin American markets for aquaculture products. Foltz et al. (1999) used discrete choice (both binary and multi-choice) techniques to show that individuals with an urban background and desiring nutritious, easy-to-prepare foods preferred fillets to whole-dressed trout. Engle and Kouka (1995) used discrete choice logit techniques to evaluate potential consumer acceptance of canned bighead carp in Arkansas. Probabilities estimated showed that canned bighead competed more favorably with canned tuna than with canned salmon. Income, region, and gender significantly affected perceptions. Gempesaw et al. (1995) used logit analysis to show that most decisions to purchase fresh seafood products for consumption were based on perceptions that products tasted good and added variety to the diet.

Quantified Anticipated Benefits

The primary direct beneficiaries of this study will be tilapia growers in Honduras and in Nicaragua. Small-scale growers, in particular, have struggled to develop markets without the benefit of comprehensive market information and of characteristics of particular market segments in Honduras and in Nicaragua. Small-scale growers have greater difficulty meeting the volume and sizing requirements of export markets and, thus, domestic markets are their primary market targets. Yet without guidance

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

based on quantitative analyses, they will be required to expend considerable resources to identify market opportunities by trial and error. The results of this study will assist them to concentrate their limited marketing time and resources on those types of market channels that are likeliest to result in sales.

The Peruvian graduate student who will work on this project as part of his thesis research will benefit from acquiring advanced quantitative analytical skills. His training, in turn, will benefit Peru by increasing its human capital and building capacity in aquaculture marketing and analysis.

The PD/A CRSP will be a secondary beneficiary because improvements in market development will result in industry expansion that will allow for greater adoption and need for PD/A CRSP research results. The overall impact of the PD/A CRSP will be greater as the industry grows in Central America.

Research Design

Location of Work: This work will be done at the University of Arkansas at Pine Bluff. This study will utilize the existing database to conduct the analyses and publish the results.

Methods

Database: Separate surveys were conducted of restaurants, supermarkets, and open-air market vendors in both Honduras and in Nicaragua in the Ninth Work Plan. Direct personal interviews were conducted in Honduras in 1999 and in Nicaragua in 2000. Complete censuses were taken of supermarkets and open-air fish market vendors in both countries. In Honduras, random samples were taken of restaurants in Tegucigalpa and San Pedro Sula (the two main urban population centers in Honduras) and in selected small rural towns. Small rural towns were selected along the primary route from north to south through the country to collect data along a possible gradient of preferences between the Pacific and Atlantic coasts. Honduras is the only Central American country with good access between the two coasts where this might be possible. Additional towns that were large enough to be included on maps and located to the east and west of the Tegucigalpa-San Pedro Sula highway were included. In all, the following small rural towns were included in the survey: Catascamas, Siguatepeque, Santa Barbara, Comaguela, Lago de Yojoa, Choluteca, Puerto Cortes, Juticalpa, Comayagua, La Paz, Santa Maria del Real, and Campamento La Lima. In Nicaragua, interviews were completed in Managua, Los Pueblos, Masaya, Granada, Boaco, Jinotega, Matagalpa, Esteli, Chinandega, Leon, and Rivas. Sampled restaurants were selected from a list of restaurant telephone listings. Fast-food eating establishments, bars, cafes, and Chinese restaurants were excluded from the restaurant survey; only full-service restaurants were represented. Supermarkets represented in the survey excluded convenience stores. In the fish markets, only those vendors with a market stand within the market were interviewed. The response rates were very high (99 to 100%) for the surveys. This is likely due to the novelty of marketing surveys in Central America. In all, there were 73 and 118 completed questionnaires for restaurants, 54 and 53 completed questionnaires for supermarkets, and 66 and 106 completed questionnaires for open-air fish market vendors in Honduras and Nicaragua, respectively.

The database developed in the Ninth Work Plan contains data on sales, supply, attitudes, and socio-demographic and economic characteristics. Sales data include information on quantities of fish and seafood species and product forms sold per week, peak seasonal demand periods, quantities of tilapia sold by product form, prices of fish and seafood products, prices of tilapia and competing freshwater fish species., and for those not selling tilapia, information on the reasons that they do not sell tilapia and the likelihood that they will begin to sell tilapia the next year. On supply issues, the database includes the primary sources of supplies of fish and seafood in general and of tilapia in particular, the principal means of transporting fish and seafood and associated costs. Additional questions were asked related to the consistency of supply of tilapia and of other similar freshwater cichlid fishes in Central America.

Respondents to the survey were presented with statements related to a variety of attributes of tilapia. They were asked to assign a value from 1 to 10 in response to statements concerning each attribute. A score of one represented complete disagreement with the sentence and a score of 10 represented complete agreement. In that way, we measured the perceptions of buyers towards tilapia and seafood characteristics. The statements included responses to the following attributes: flavor, odor, size, price, nutritional value, quality, customer preference, reliability of supply, availability of tilapia, variety, and preparation.

Socio-demographic and economic characteristics data include the following: customer income, weekly sales, clientele type, age, location, size of store, education, years in business, and seating capacity and cuisine type for restaurants.

Quantitative Analysis: Discrete choice (logit/probit) regression models will be developed to quantify the effects of socio-economic and demographic variables on the decision to sell or not to sell tilapia and on the likelihood of the restaurant, grocery store, and market stands to sell tilapia the next year, respectively.

Development of regression models depend on the type of dependent variable. Since questions in the survey related to fish sales decisions are consistently associated with discrete-valued answers, we expected all dependent variables to be discrete values. For example, questions related to the decision to sell (or not to sell) tilapia involve a one or zero answer, where one (zero) represents that the seller had chosen to sell (or not sell) tilapia. Questions related to a restaurant's willingness to put tilapia on the menu have answers coded in multi-valued discrete numbers, i.e., not willing (code = 0), willing (code = 1), very willing (code = 2), and a multi-choice ordered dependent variable will be used.

Logit models explain the variation of y and assume the existence of an underlying latent (or unobservable) variable which exhibits its value through an observed dependent variable. These models explain the variation of y, using the variation of independent variables which are postulated to have a causal linkage with the dependent variable. The types of independent variables used will include: location of market (region/size of town or city), market outlet descriptors (size, clientele characteristics, cuisine type, experience selling seafood, and attitudes and preferences). Logit model regressions are run by maximizing the likelihood of the respective models with respect to the choice of coefficients in such a manner that the probability of observing the given values of the dependent variable are as high as possible. A likelihood function can be developed from the product of different probabilities for the dependent variable.

Regional Integration

The regional plan for Central America refers specifically to planning and implementing marketing research activities in the region and for cooperation with the proposal PI. Research needs for tilapia culture in Central America refer specifically to market development.

Schedule

7/1/01	Initiate project.
4/30/02	Complete discrete choice analyses of restaurant data, Honduras and Nicaragua.
6/30/02	Complete discrete choice analyses of supermarket data, Honduras and Nicaragua.
12/30/02	Complete discrete choice analyses of open-air fish market vendors, Honduras and Nicaragua.
4/30/03	Final report submitted.

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Cost Containment Options for Tilapia Production in Central Luzon, Republic of the Philippines

Product Diversification Research 2 (10PDVR2)/Experiment/Philippines

Collaborating Institutions
Central Luzon State University, Philippines
Remedios B. Bolivar

Florida International University
Christopher L. Brown

Objectives

1) To conduct farm trials and production cost analyses, designed to test the hypothesis that the two methods of reducing rations resulting in increased profit (under the Ninth Work Plan) have an additive effect.
2) To conduct farm trials and production cost analyses, designed to determine the efficacy of increasing grow-out efficiency by way of reducing rations by feeding on alternate days.
3) To integrate economic figures generated in the first two objectives in the course of analyses of production costs in order to develop a comprehensive analysis of the costs and benefits of grow-out options. The goal of this objective will be to test whether cost containment occurs.

Significance

Regional growth in the tilapia farming industry and increasing stature of the Freshwater Aquaculture Center at CLSU have gone hand in hand. The proposed investigation continues a line of research initiated at the Asian Institute of Technology in the early 1990s and adapted for the benefit of farmers in the Philippines, at Central Luzon State University, more recently.

Regional aquaculture research attention has maintained a focus on increased profitability, as opposed to intensification or maximization of yield without regard for cost (see Diana, 2000). Studies conducted by the PD/A CRSP earlier in Thailand suggested that supplemental feeding of tilapia in fertilized ponds can induce growth advantages over feeding or fertilization alone (Diana et al., 1994), and that the timing of feeding can be manipulated to the advantage of farmers in Thailand (Diana et al., 1996) and in the Philippines (Brown et al., 2000). The advantages of either delayed feeding or feeding a sub-satiation level diet are realized in the cost savings that occurs with the reduction in the amount of processed feed that must be purchased by farmers. Our analysis, based on trials at seven participating commercial farms in the Philippines, showed that delayed feeding led to reductions in feed costs amounting to approximately 37% without a significant loss of crop value at harvest (Brown et al., 2000). Similar reductions were seen by reducing the amount as opposed to the timing of feeding; the first objective above is intended to determine whether these effects are additive.

The determination of feeding strategies based on mathematical and economic models can be rather complex (Cacho, 1993). It is not known whether the reduction of food costs without a net reduction in crop yield is a result of more efficient food consumption (i.e. lack of waste), better food utilization (increased food conversion ratio) or both. It has also been argued that optimal feeding levels should be below the level supporting maximal growth on the basis of water quality concerns; regularly feeding to the point of satiation increases the risk of waste, food decomposition, and compromised fish health (Hatch and Kinnucan, 1993). The modeling of optimal feeding regimes is based on a mix of observations and assumptions. One vitally important component in these calculations—feed consumption efficiency—is quite difficult to quantify in aquatic animals (Lovell, 1989). Most often, decisions about the amount to feed are left to chance or the instincts of the farmer.

The proposed experimentation will evaluate the possible additive effects of two methods of cost reduction—delayed onset of feeding and feeding at sub-satiation levels. Each has been shown independently to reduce the cost of grow-out, and it is possible that a further cost reduction can be gained by combining the two strategies. The delayed onset of feeding probably causes fingerling fish to depend more, or longer, on the plankton blooms or other natural productivity of fertilized ponds. These farm trials will provide a clear indication of whether reduction of food expenditures at two stages (delayed onset and reduced ration) present another cost-effective feeding option for farmers, or whether this approach compromises yield or product quality to an undesirable degree.

A second set of farm trials will test the provision of prepared feeds on alternate days, in order to determine the net effect that this strategy will have on grow-out efficiency and net profit. The combined significance of the proposed farm trials is the comparative evaluation of a range of alternative methods of cost reduction under semi-controlled conditions (commercial aquaculture). Economic analysis will be used to develop an overview of the integrated capacity of these methods to reduce production costs.

Quantified Anticipated Benefits

The proposed field research will take place on at least nine commercial farms and will clarify the relative benefits of practical feeding strategies for tilapia grow-out. We will build on the successful demonstration of two such feeding strategies with evaluations of a third strategy and a combination of the two established methods.

Fish farmers in the Philippines and in Central Luzon in particular readily adopt appropriate new technology. Tilapia is a staple in the Philippines, and tilapia farming is carried out on a huge scale. Tilapia contributes heavily to Philippines fish farming productivity, which currently ranks fifth world-wide (Anon., 2001). A modest reduction in the cost of production when carried out on such a large scale could translate into a significant economic boost.

Research Design

Location of Work: As in recent farm trials, tilapia fingerling will be obtained under agreement with the Genetically Improved Farm Tilapia (GIFT) Foundation. Our past arrangement has been to obtain these fast-growing tilapia at a nominal cost in exchange for the public exposure that is gained in the course of on-farm experimentation. We are currently negotiating a more formal agreement with the GIFT Foundation, expected to result in a signed Memorandum of Understanding defining the terms of our cooperation with them.

Grow-out experiments will be carried out at farms within driving distance of the Central Luzon State University campus in Muñoz, Nueva Ecija province, Central Luzon. Farmers will be selected on the basis of their willingness to follow prescribed protocols, the availability of identical or nearly identical ponds for side-by-side comparisons of treatments, their assumption the risks inherent in the unpredictable outcome of an experimental trial, and proximity to the Freshwater Aquaculture Center. We have a solid working relationship with nine area farmers who have expressed interest in continued cooperation with our project, and we will also consider new locations for the proposed farm trials.

Methods: Sex-reversed, genetically selected tilapia fingerling will be obtained from the GIFT Foundation and stocked into ponds on participating farms at an initial density of 4 fish per m^2. The natural productivity of the ponds will be promoted with the addition of chemical fertilizers at the rates of 28 kg N per ha per wk and 5.6 kg P per ha per wk. Feed prepared at the Freshwater Aquaculture Center, consisting of 67% rice bran and 33% fish meal will be used for these studies. Fifty fish will be sampled every two weeks for weight determination. Undergraduate and graduate students will be involved in these studies.

In the first trial, both treatment groups will be fed beginning on day 75, as in our previously reported experimentation (Brown et al., 2000 and ms in preparation). One group will be fed to satiation and the other will be fed to 67% of satiation (rations to be based on experimental determination). At least one pond consisting of each of the two treatment groups will be monitored at each participating farmsite; in each case growth rates, yields, and uniformity of fish will be assessed. Growth data will be subjected to Paired T-tests in order to test the hypothesis that the use of a reduced ration has no effect on growth rate in a delayed feeding paradigm.

Farmers will be asked at the time of harvest for any additional observations they may have on fish health, behavior, or quality. Crop value and production costs will be calculated in order to determine the net production cost for each of the two feeding strategies. These data will be compared with the cost analyses generated in the course of the Ninth Work Plan experimentation.

The second set of farm trials will follow the same general guidelines as the first, in terms of location, stocking densities, nutrient inputs, sampling, etc. except that a different set of feeding schedules will be used. In this trial, the same feed (67% rice bran and 33% fish meal) will be given to fish, but the comparison will be between fish fed to satiation daily and fish fed the same amount on alternate days. We will test the null hypothesis that this 50% reduction in ration does not disrupt growth rate, again using the Paired T-test.

Economic data from the pond trials will be pooled to examine the productivity of ponds under the various production regimes, in terms of potential net profit per hectare. Reducing feed costs is a primary goal of these studies, but the efficacy of reduced-feeding paradigms is only demonstrable in terms of crop yields and values. Economic analysis will be carried out using the same approach we have used in the past two years. Specifically, we will calculate the cost of production as a consequence of varying the feeding schedule, based on the use of feed costs as the independent variable and yield in terms of kg per hectare as the dependent variable. Data collected at the time of harvest will also allow us to make a determination about uniformity (variance in body mass) and % survival rate (number harvested/number stocked). Production costs will be presented as both Philippine pesos and US$ per kilogram. This enables us to make a direct comparison of control and experimental treatments, and a reduction in the production cost among the experimental groups would be interpreted as indicative of cost containment. Wholesale crop values will be used to calculate the approximate profit margin for each feeding paradigm, on both a pesos per hectare and US$ per hectare basis. In addition, qualitative indices of crop value, such as uniformity, subjective comments of farmers, observation of deformities if any, etc. will be noted. This method does not figure in labor costs, since the amount of time required to prepare feeds, weigh them, and feed ponds are difficult to measure accurately and the cost of labor on a family farm is a nebulous concept.

Regional Integration

The tilapia is a crop with established and growing importance in Southeast Asia, and the generation of new strategies for tilapia culture could have an economic impact throughout the region. Based on our most recent experience, we anticipate that the proposed studies will provide insight into the suitability of a variety of cost-cutting options available to farmers—not so much in the form of a dogmatic paradigm to be followed, but a series of choices and expected benefits available for their use as they see fit, and depending on the circumstances on their farms. Central Luzon State University is involved in training of students from throughout the region, and as students and trainees return to their home countries, technology generated in the course of this project will go with them.

We also recognize and plan to take advantage of the fact that electronic dissemination of technical information is occurring at an accelerating pace. Within the past few years, Central Luzon State University has been revolutionized by electronic communications. At the outset of our collaboration in 1997, access to telephones on campus was extremely scarce and a typical exchange of messages by fax involved a significant degree of uncertainty. In 2001, it is commonplace for CLSU students to transfer text files to each other by cell phone. Technical information is being posted on websites, and distributed in electronic publications. The frequency of access to this sort of information is easily quantified. We plan to post and regularly update a project website, and anticipate that as we build our knowledge base of information on the various means of providing substantial cost-savings, the interest in and frequency of access to this website will increase, and will include farmers beyond Luzon and the Philippines.

Schedule

Year 1

Date	Activities
7/1–8/01/01	1) Identify participating farmsites, 2) Outline experimental variables and schedule, 3) Obtain sex-reversed tilapia fingerlings from GIFT Foundation for distribution 4) Establish website containing project information, updated regularly
9/1–9/30/01	1) Stock fingerlings into fertilized ponds; 2) initiate sampling at two-week intervals
11/15–12/15/01	Initiate feeding (75 days post stocking)
3/1–3/31/02	Harvest first farm trial, conduct cost analysis

Year 2

Date	Activities
7/1–8/01/02	1) Meet with participating farmers to review Experiment 1 and outline experiment 2, 2) Obtain sex-reversed tilapia fingerlings from GIFT Foundation for distribution.
9/1–9/30/02	1) Stock fingerlings into fertilized ponds; 2) Initiate sampling at two-week intervals
10/15–11/15/02	Initiate feeding (35 days post stocking), daily feeding (control) and alternate-day (expt'l) groups.
3/1–3/31/03	Harvest second farm trial, conduct production cost analysis
4/10/03	Host workshop to disseminate information to farmers
4/30/03	Submit final project report

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Transfer of Production Technology to Nepal for Nile Tilapia, *Oreochromis niloticus*

Product Diversification Research 3 (10PDVR3)/Activity/Nepal and Thailand

Collaborating Institutions
Asian Institute of Technology, Thailand
Amrit Bart

Regional Agricultural Research Station, Tarahara, Nepal
A.K. Rai

The University of Michigan
James S. Diana

Objectives

1) Select one broodstock group of Nile tilapia from AIT and carry out on-station production trials (fertilization only, fertilization and feeding) in Nepal (Year 1).
2) Introduce hapa-based sex-reversed tilapia seed production technique - On-station trial (Year 2).
3) Carry out farm growth trials of above stock (mixed-sex and sex-reversed) utilizing CRSP best management protocols and experience in southern and central Nepal (Year 2).

Significance

The CRSP studies have made significant contributions to determining the optimal fertilization regimes for warm and cool weather conditions (Brown et al., 2000; Veverica et al., 2001), feeding (Diana et al., 1996), production of monosex populations (Green and Teichert-Coddington, 1994; Gale et al., 1999; Phelps and Warrington, 2001) and polyculture with other species (Szyper and Hopkins, 1997). The tilapia production industry has benefited a great deal from these and other studies, particularly in Southeast Asia. Problem-based research studies at AIT in Thailand has played a critical role in providing technical and research support that has facilitated the industry to expand in many different areas.

Additionally, we have found that low-input tilapia production has especially benefited resource poor farmers in the Southeast Asia. Small-scale tilapia growers also tend to consume their own fish, thus increasing high-quality protein in household diets. Unfortunately, some of the poorest countries in Asia, including Nepal, have not received direct benefits of PD/A CRSP presence in Thailand. Although fish is highly desirable in Nepalese diet, per annual consumption is only 1 kg per person (Edwards, 1998). Nepal, one of the poorest nations suffers severe malnutrition problems especially among young children. Tilapia, lauded as a low input aquaculture species, has the potential to provide cheap protein in children's diet if the culture technology is developed to benefit small-scale farmers.

Lack of tilapia production in Nepal is partly due to previous government policy to not introduce exotic species that might have negative impact on the local biodiversity and indigenous species. Nile tilapia *Oreochromis niloticus* was first transported to Nepal in 1985 and held in various government research stations for research and development purposes (Pullin, 1986; Singh, 1995). The origins of these strains are unclear and further development work has not been done on tilapia culture. The government has now realized that there is an increasing interest among farmers to raise tilapia, which is indicated by import of seed from neighboring India. A number of farmers have stocked this species in their ponds and hapas. This uncontrolled and unchecked introduction of tilapia is seen as having a negative impact on small-scale farmers' livelihood because the fish they use to begin aquaculture systems are of poor quality. The Fisheries Research Division, Godawari of the National Agricultural Research Council (NARC) has been charged with the responsibility to develop a production package that includes importation of appropriate strains, seed production using modern techniques and grow-out technology that is suitable to low input conditions of southern and central Nepal.

PD/A CRSP at AIT has many years of research and experience in pond/hapa-based tilapia production, which could be reasonably adapted to sub-tropical conditions of Southern Nepal. This activity proposes to assist in transfer of PD/A CRSP developed technology on tilapia culture to Nepal through on-station trials with feeding and fertilizing, as well as production of sex-reversed and mixed-sex seed in hapas.

Anticipated Benefits

Successful introduction of tilapia culture techniques is expected to:

1) Increase fish production and protein levels in the household diet of rural poor;
2) Prevent uncontrolled transport of low quality seed from India; and
3) Assist in identifying research needs and facilitate future CRSP activities in Nepal.

Activity Design

Location: NARC research facilities, Tarahara, Nepal

Methods

Production Trials

Earlier trials in PD/A CRSP by Diana et al. (1996) demonstrated that initiation of feeding after 80 days produced the same yield as feeding from day zero. Fish given feeding after day 80 also reached the same expected size, with some time delay. An on-farm trial was conducted by Brown and Bolivar (2000) in the Philippines based on Diana et al. (1996) report and observed similar results, particularly when economic returns were considered. We intend to follow similar trial in Nepal using on-station facilities. On station trials will be conducted on Improved and selected lines (GIFT 6^{th} generation selects) of tilapia.

Nile tilapia fingerlings (35 days old, 1,200 total) will be transported to Nepal where they will be placed in several 50-m^2 ponds at 4 fish/m^2. Half of the ponds will be fertilized weekly with urea ammonium sulfate at a rate of 28 kg N ha^{-1} wk^{-1} and 5.6 kg P ha^{-1} wk^{-1}. The other half of the ponds will be fertilized and fed diet starting on the 80^{th} day. The diet will consist of 67% rice bran and 33% fishmeal applied at 3% BWD until harvest. A sample of 50 fish will be removed every two weeks to measure average weight of fish. Water quality will be monitored according to standard methods (APHA, 1980; PD/A CRSP, 1992).

Fish will be harvested after 180 days. Variables measured will include total number of fish and bulk-weight. Final total feed given, mean weight, daily weight gain, gross yield and survival rates will also be calculated. Data will be analyzed by paired t-test.

Seed Production Trials

Production of mixed-sex and monosex fry and fingerlings will be based on AITs hapa-based model. Broodfish will be selected from the pool of fish in the above trial and stocked in hapas in 200-m^2 ponds at a rate of 4 fish/m^2. Broodfish will be fed 67% rice bran and 33% fishmeal at 2% BWD. Female fish will be checked every 4-5 days for eggs. Fertilized eggs will be pooled by development stages and separated for incubation.

A low-cost flow-through incubation tray system will be constructed to allow sufficient flow rate to keep fertilized eggs well oxygenated and moving (Little 1986). Hatched fry at three days after yolk sac absorption will be divided into two groups. One group will be fed with commercially available 17α-methyltestosterone coated diet and the other group will receive no hormone. Larvae will then be fed with 67% rice bran and 33% fish meal at 5% BWD up to 15 days and 4% up to 30 days. Both groups will be raised up to 120 days and sex ratios compared between these two treatments. Additionally, fecundity, fertilization rate, hatch rate, and survival rate data will be assessed.

Economic profitability will be compared between fertilized pond and fertilized + fed pond.

Regional Integration

This initial activity is expected to lead to many problem-based and demand driven research in the region. Successful introduction and integration of tilapia culture may be used as a model for other poor countries with similar environmental conditions such as north India, Bhutan, Bangladesh, and Myanmar.

Schedule
Production trial: August 2001 to March 2002
Seed production trial: April 2002 to January 2003
Report submission: 15 March 2003

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POND DYNAMICS/AQUACULTURE
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Date of Release: 15 February 2001

RESTRICTED REQUEST FOR PROPOSALS FOR THE PD/A CRSP TENTH WORK PLAN

Issued by the Management Entity of the Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis, Oregon, USA

Proposals are encouraged from PD/A CRSP member institutions and from former CRSP institutions participating under the current Grant (USAID Grant No. LAG–G–00–96–90015–00). Proposals from institutions without a present or former affiliation with the PD/A CRSP are ineligible for award of this grant. This restriction does not exclude Host Country (HC) institutions, as eligible US universities may propose to collaborate with HC institutions not mentioned in the CRSP Grant.

IMPORTANT DEADLINES

26 January 2001	Pre-RFP notification
15 February 2001	CRSP Management Entity (ME) releases RFP
2 April 2001	Proposals due at the ME
9 April 2001	PMO sends proposals to reviewers
8 May 2001	Reviews are due at the PMO
25 May 2001	PMO sends reviews to the Technical Committee (TC) and Board of Directors
11 June 2001	TC and Board recommendations to the ME
29 June 2001	ME evaluation and selection of proposals
2 July 2001	ME notifies Principal Investigators (PIs) and awards subcontracts

The CRSP ME reserves the right to modify this schedule as necessary to ensure that standards of fairness and accuracy are met. PIs will be notified if the award decision will be delayed.

A. ELIGIBILITY

The following US universities are eligible:

Auburn University
Florida International University
Michigan State University
Ohio State University
Oregon State University
Southern Illinois University at Carbondale
University of Alabama at Birmingham
University of Arizona
University of Arkansas at Pine Bluff
University of California at Davis
University of Delaware
University of Georgia
University of Hawaii
University of Michigan
University of Oklahoma
University of Pittsburgh
University of Texas at Austin

PD/A CRSP Tenth Work Plan RFP

B. Introduction & Purpose

The CRSP ME is inviting proposals for the final work plan of the current CRSP grant. The purpose is to complete the research outlined and approved in the CRSP Grant. Proposals are therefore solicited for continuing projects in promising areas not yet fully completed and for "gap" projects areas identified in the Grant but not undertaken in the Eighth and Ninth Work Plans.

The PD/A CRSP was awarded a five-year grant (*Continuation Plan 1996-2001*) by the United States Agency for International Development in August 1996. The grant is expected to be extended two additional years, allowing for this Tenth Work Plan. The goal of the grant is to provide a basis for improving the sustainability of aquaculture production systems. The *Continuation Plan* identified constraints to aquaculture development and provided a Results Framework—a cohesive, integrated approach—to address these constraints. (See the PD/A CRSP RFP website at <http://pdacrsp.orst.edu/rfp> for these grant excerpts and for all forms, instructions, and background materials referenced in this RFP.) The framework uses two building blocks to identify research priorities: research in production systems and capacity building via research support. Research in production systems is organized into three research areas: production optimization; environmental effects; and social and economic aspects. Each research area is further subdivided into specific research themes (summarized in section C.3). CRSP research is assisted by research support functions, which build capacity through educational development, information, networking, and database management.

CRSP research addresses research priorities in four geographic regions: Southeast Asia, Africa, Central America, and South America. Prime sites operate as regional focal points for research and outreach endeavors. They coordinate their investigations with those at companion sites in order to extend CRSP results. Current "Prime Sites" are Honduras, Kenya, Philippines, Peru, and Thailand. Current regional sites are Mexico and Malawi. Regional aquaculture research priorities are described in Regional Plans (RFP website: Regional Plans).

The following types of proposals are invited:

- **Regional proposals**
  Investigations in regional proposals ideally address the issues identified in existing Regional Plans by employing a systematic, multi-disciplinary, integrated approach and using research themes specified in the *Continuation Plan*. These investigations are to be conducted at a current prime site or at current and/or new companion sites (as identified in the Regional Plans or *Continuation Plan*). Investigations in regional proposals must identify at least one HC PI for each investigation proposed. Note that new companion sites may be identified for new investigations under the Tenth Work Plan (see RFP website for Site Selection Criteria Guidelines and for USAID-eligible countries).
- **Cross-cutting research proposals**
  Cross-cutting investigations address specific research themes identified in the *Continuation Plan.* Cross-cutting research is not necessarily conducted at one specific site and ideally attends to issues of regional significance and integration with regional work plans. Cross-cutting work plans must identify at least one HC PI for each investigation proposed.

Proposals may contain more than one investigation. An investigation is either an experiment, a study, or an activity (see definitions in C.4). Proposals must not contain more than one activity.

PD/A CRSP Tenth Work Plan RFP

C. Background Information

1. Previous Work Plans

The Eighth and Ninth Work Plans describe research and research support efforts for the period 1996 to 2001. Investigations were implemented in the following countries: Honduras, Kenya, Peru, Philippines, Mexico, Nicaragua, Guatemala, Panama, Malawi, and Thailand. Information on these investigations may be obtained from CRSP Annual Administrative and Technical Reports for 1997, 1998, 1999, and 2000 (RFP website: Annual Reports). The CRSP Eighth and Ninth Work Plans and addenda (RFP website: Work Plans) provide additional background on these investigations.

2. Previous Allocations to Research Areas, Research Themes, and Regions

Research described in the Eighth and Ninth Work Plans addressed each of the three CRSP research areas. Most of the research effort was devoted to investigations in production optimization (51%), followed by research in social and economic aspects (36%), and environmental effects (14%). Several research themes were not fully addressed in the first two work plans (Table 1). The allocation of research effort to the four regions identified in the Grant is also shown (Table 2).

Table 1. Percent Allocation per Research Area and Research Theme for the Eighth and Ninth Work Plans Combined

Research Area	Research Theme	Proposed percentage	Percentage funded to date
Production Optimization	Pond Dynamics	28	15
	Feeds and Fertilizers	20	23
	Reproduction Control	28	18
	Aquaculture Systems Modeling	9	3
	New Aquaculture Systems/New Species	15	12
Environmental Effects	Effluents	43	54
	Appropriate Technology	22	16
	Responsible Science Policy	2	0
	GIS: Planning, Policy, Global Data Analysis	33	0
Social and Economic Aspects	Marketing and Economic Analysis	18	8
	Adoption and Diffusion	20	37
	Decision Support Systems	20	18
	Regional Analysis/Human Environment Int	11	0
	Food Security	22	0
	Product Diversification	9	0

Table 2. Percent Allocation per Region for the Eighth and Ninth Work Plans Combined

Region	Sites in Region where investigations were undertaken from 1996-2001	Proposed percentage	Percentage funded to date
Africa	Kenya, Malawi	30	23
Asia	Thailand, Philippines	26	20
Central America	Honduras, Mexico, Nicaragua, Panama, Guatemala	28	20
South America	Peru	16	9

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3. Summarization of Research Themes. See the RFP website (Research Theme Descriptions) for the full versions of research themes from the *Continuation Plan*.

Research Area: Production Optimization

Pond Dynamics
Pond dynamics refers to the dynamic processes occurring in a fish pond, particularly interactions among nutrients, primary and heterotrophic productivity, and fish yield. However, water quality imbalances that have their origin in interactions between the pond soil and water still can not be mitigated effectively. Studies to determine the characteristics of the pond sediment and the influence of these characteristics on the exchange of substances between soil and water are critical to the development of effective pond management techniques. The contribution of heterotrophic organisms to pond productivity and the potential to harness these pond resources also remains unclear.

Feeds and Fertilizers
Continued development of commercial aquaculture demands additional research to develop more sustainable intensified production practices including cultured species, maintenance of aerobic conditions, and management of nutrient inputs, specifically feed. The PD/A CRSP pond management experiments conducted to date have resulted in the development of strategies for various levels of farming intensity. Further research is required for intensified pond management strategies in order to optimize resource utilization (e.g., formulated feeds, water, stocking rates). The proposed research will contribute to the development of practical guidelines for significantly improved management of ponds due to enhanced fertilization efficiency, maintenance of water quality, and improved resource utilization. The recovery of nutrients usually lost in water and sediment will reduce nutrient levels of pond water discharge into receiving waters.

Reproduction Control
In countries with semi-intensive to intensive aquaculture systems, all-male tilapia culture may be preferable because of the demonstrated superior growth characteristics of male tilapia. The standard method for producing all-male populations (dietary MT treatment) has been investigated at CRSP sites in Africa, Asia and Central America. Therefore, the proposed PD/A CRSP research will focus studies to optimize dietary MT treatment efficacy and safety. The effect of humidity on diet storage and of water temperature on dietary hormone treatment will also be investigated. Furthermore, development of a safe-handling manual for male-producing hormones and investigations on the fate of MT released from the diet and/or fish into pond water will address worker and environmental safety issues. Research may be conducted on the fate of MT and its metabolites in the fish and on the establishment of MT dosages safe to tilapia. These experiments will be conducted using FDA-approved methods and after consultation with the National Investigational New Animal Drug Coordinator to ensure data acceptability for FDA consideration.

Aquaculture Systems Modeling
Current water quality models need improvement before they can be used for assessing the impacts of water quality on pond production, effluent quality, and the environment. A key issue in this assessment is quantifying the role pond sediments play in overall water quality dynamics. The proposed research will generalize the results developed in other studies into research and management models focused on pond soil/sediment characterization and soil-water interactions. Additionally, models previously developed under CRSP direction may be extended, calibrated, and validated to consider integrated agricultural/aquacultural systems and then implemented within the decision support system framework of POND©.

New Aquaculture Systems/New Species
Due to negative impacts that introduction of non-native species may cause, the need exists to evaluate the aquaculture potential of local and native species and to develop new culture technologies. The PD/A CRSP proposes to conduct a comprehensive analysis of various aspects of indigenous fish culture. To secure a sustainable source of animals for culture, a broodstock program must be implemented for any species cultured. In addition, research on broodstock nutrition and larviculture will be undertaken to establish procedures for producing sustainable populations of high quality fish for culture. A second objective is to assess the economic feasibility of indigenous species by using production information and market surveys.

Research Area: Environmental Effects

Effluents and Pollution
The discharge of nutrients and sediments in effluents from intensive aquaculture operations has been shown to contribute significantly to eutrophication in receiving waters. Several developing countries are experiencing problems with pond effluents, and a few developing nations are requiring discharge permits for aquaculture operations. Also, as restrictions are placed on the discharge of pond effluents, in-pond or effluent treatment strategies might be required. Water use in aquaculture, relative to other potential uses of water, is also a major environmental concern, especially in arid and semi-arid climates. Results derived from research on the impacts of pond effluents on receiving waters will provide guidance in policy formulation to resource managers. Development of alternative production and harvesting strategies, which result in reduced discharge of pond nutrients, will benefit aquaculture in the US as well as in developing countries.

Appropriate Technology
Appropriate technologies address not only environmental constraints, but also improve engineering designs to target local social conditions. Therefore, in response to similar situations of difficulties in direct technology transfer, the PD/A CRSP will translate

theoretical insights from global research activities into practical solutions to local needs and constraints in order to maximize impact of research results.

Responsible Science Policy
The PD/A CRSP proposes to develop policies for the use of exotic species, biotechnology, pharmaceuticals, and other issues of concern when doing international research. These policies will govern research activities at all PD/A CRSP sites, and provide a model for HC institutions that wish to develop their own policies. The proposed rules will be cognizant of policies developed by international organizations, as well as U.S. and HC regulations, and will provide PD/A CRSP researchers and management personnel with detailed instructions on this issue.

GIS: Planning, Policy, and Global Data Analysis
One of the major constraints to strategic planning for sustainable aquaculture development is the lack of easily accessible information and analysis tools that can be used to examine different production scenarios and their potential social, economic, and environmental impacts. The PD/A CRSP Central Database is a repository of standardized data that can be evaluated and incorporated into a regional information base such as a Geographic Information System. The PD/A CRSP will develop a regional information base as well as qualitative and quantitative models that will enhance the planning process and improve the policies that are the result of such a process.

Research Area: Social and Economic Aspects

Marketing and Economic Analysis
Future recommendations will incorporate economic factors and analyses to ensure that farm income and household food security are impacted positively. Economic analyses to determine the impact on net farm income of various production factors such as input levels, input prices, and value of marketed products will indicate which production systems have the greatest positive economic impact. Production, financial, and marketing risk affect both the overall profitability of a particular enterprise and the likelihood that farmers will implement a new technology. Analytical techniques exist to quantify the effects of the risks involved in different production technologies on farm profits and on the overall economic sustainability of an enterprise. Market analysis will identify the most appropriate and profitable geographic and demographic markets, as well as the segments within those markets for different aquaculture products. Cost analyses of processing, transportation, and storage alternatives will indicate the most profitable strategies and identify critical economic control points.

Adoption/Diffusion
It is important to understand the role of the aquacultural enterprise in the farm household, the infrastructure for marketing and distribution, and the place of fish in the dietary regime of the population. The impacts of a new enterprise on women, the effects of the fish pond on other activities, the disposition of harvests, harvest proceeds, and post-harvest use of fish are also to be considered. Results of the proposed studies will provide important insights into the integration of PD/A CRSP technologies into HC farming systems and will support recommendations for improving the profitability of PD/A CRSP management systems. Evaluations of farm-level use of these technologies will indicate the appropriateness of PD/A CRSP research.

Food Security
The proposed research will provide specific information on the food security needs of potential project beneficiaries at nutritional risk, including both producer households and consumers near PD/A CRSP project sites, in order to assist in the setting of priorities for project activities. It will also assess the probable impact of increasing the availability of fish on the food security of poor rural and urban consumers, develop and implement a system of food security and nutrition monitoring to complement on-farm testing and farming systems research at PD/A CRSP sites, and assess the impact of increasing aquaculture activities on the access of rural populations to other food resources.

Decision Support Systems
Computer models facilitate the exploration of planning and management options that address biological, chemical, physical, and economic issues relevant to pond production and optimization. The proposed research will further improve available decision support and modeling capabilities, and will enable managers to evaluate facility performance based on economic performance and environmental constraints.

Regional Analysis: Human-Environment Interactions
Constraints to aquacultural development are often social and economical; e.g.. governmental regulations, availability of infrastructure (roads, markets, refrigeration), access to advertisement opportunities, consumer expectation and demand, culturally acceptable forms of producer organizations. In order to create successful national or regional aquaculture development, these constraints and their relative importance need to be known to decision-makers. Proposed research would develop an information base to assess aquaculture potential and provide recommendations to alleviate the most limiting constraints.

Product Diversification
Despite PD/A CRSP efforts in developing production guidelines and increasing fish yields, rural farmers may still face economic failure because the short life-span of fresh fish in tropical countries without adequate refrigeration hinders the farmers' ability to sell their products fresh to distant markets. Often, the farmers' only other choice is to sell the fish live from the ponds during harvest. Through post-harvest research, the PD/A CRSP can further aquacultural development by enabling farmers to successfully market new products to a wider audience.

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4. Definition of Investigation, Experiment, Study, and Activity

An investigation may be an experiment, a study, or an activity.

- Experiment: A scientifically sound investigation that addresses a testable hypothesis. An experiment implies collection of new data by controlled manipulation and observation.
- Study: A study may or may not be less technical or rigorous than an experiment and may state a hypothesis if appropriate. Studies include surveys, database examinations, most modeling work, and collection of technical data that do not involve controlled manipulation (e.g., collection and analysis of soil samples from sites without having experiments of hypothesized effect before collection).
- Activity: An activity requires staff time and possibly materials but does not generate new information like an EXPERIMENT or a STUDY. Conference organization, training sessions, outreach, and transformation and dissemination of information are examples of activities.

D. Twelve Major Programmatic Considerations for Award of a CRSP Project

1. Proposals will ideally present a multi-disciplinary, cohesive approach to aquaculture research, outreach, and development in selected HCs.

2. Proposals that address existing gaps in the current CRSP portfolio are encouraged for this Tenth Work Plan. The *Continuation Plan* specifies the following distribution of research effort over the life of the grant: production optimization (47%), social and economic aspects (29%), and environmental effects (24%). Proposals are especially invited for those research themes that were not addressed in the Eighth and Ninth Work Plans (Table 1). Other research areas are also invited for the Tenth Work Plan but funding will be allocated first to those areas that have been partially completed or not completed during the Eighth and Ninth Work Plans.

3. The CRSP model requires at least 50% of project funds to be expended in or on behalf of the HC or region. Failure to allocate 50% of funding in or on behalf of HCs will disqualify the proposal.

4. Proposals must be consistent with USAIDs strategic objectives in economic growth and education; sustainable management of natural resources; and health. (See the RFP website for link to USAID.)

5. Each applying university must provide the full 25% US non-federal cost sharing required by USAID and BIFAD. HC partners are encouraged to provide matching funds but are not subject to the same federal requirements as are US universities.

6. Salary support for US PIs cannot exceed 0.15 FTE (total for all US PIs). US PIs charging any portion of salary to the CRSP award must also be serving in the capacity of major advisor to a CRSP graduate student working under an approved CRSP investigation.

7. Proposals that describe plans (or have potential) for leveraging support from other sources in furthering the broad goals of the CRSP are desired. Leveraged support is support in addition to US non-federal cost sharing funds required for award of a CRSP project and in addition to the optional HC institutional match.

8. A key consideration for award of CRSP funding is that the recipient university provide strong institutional support. The ME wants to promote the institutional sustainability of prime sites and is therefore interested in pursuing and furthering arrangements that will last long after the CRSP ceases to exist as a funded entity.

9. Collaborative efforts that involve undergraduate students, graduate students, and post-doctoral fellows are encouraged. Expatriate (US) researchers will not be funded.

10. Current CRSP researchers with incomplete Ninth Work Plan investigations will **not** be eligible to receive funding for the Tenth Work Plan until all obligations are satisfactorily met on previous work. These researchers may elect to postpone the start date of their investigations under the Tenth Work Plan. Researchers must still adhere to the schedule for responding to this RFP. Proposals starting after July 2001 must clearly specify the dates of the investigations. In no cases will investigations be permitted to continue beyond 30 April 2003.

11. Familiarity with institutions in the proposed HC and region as indicated by past relationships is desirable, as is an institutional track record of work in the proposed country.

12. Proponents from diverse backgrounds are encouraged to apply.

E. Considerations for Adding New Host Country Collaborating Researchers and Institutions

1. Proponents wishing to add a new companion site to one of the CRSP regions must include a response to the criteria for companion sites outlined under Site Selection in the *Continuation Plan* (RFP website: Site Selection Criteria). New prime sites are not being considered for the Tenth Work Plan. (See also the RFP website for USAID-eligible countries.)

2. Upon receipt of a CRSP award, the recipient university may be required to enter into Memoranda of Understanding (MOU) with institutions at companion sites. MOUs must provide the opportunity for other CRSP projects to function under the authority of the agreement.

F. Programmatic Considerations for Fostering Respectful Partnerships

1. Proposals that foster linkages with organizations including US minority institutions, non-governmental organizations (NGOs), private voluntary organizations (PVOs), national agricultural research institutions, other CRSPs, and international centers are desired. Linkages strengthen the network for aquaculture research and development and improve chances of a project's ability for achieving positive impact.

2. Proposals that link HC researchers from one CRSP site to another are encouraged.

3. Once funded, PIs will be expected to cooperate with other CRSP PIs involved in research in each of the CRSPs regions. Regional plans have been developed by the CRSP; these plans may be modified as priorities and needs change (RFP website: Regional Plans).

4. US PIs must be willing to actively pursue an open, respectful, and collegial relationship with their counterpart PIs from HC institutions. This means that US and HC PIs will share in

budgetary decisions and overall priority setting, as well as in other collaborative activities related to the CRSP. Proposals, work plans, and budgets must be developed collaboratively among HC and US researchers.

5. US PIs must actively establish an effective working relationship with the ME and with CRSP Research Support Projects (Database Management Project; and Information Management & Networking Project), and also with USAID in the HCs and adjacent region.

6. Proposals must contain Letters of Interest from prospective US and HC partners.

G. Technical Considerations for Award of a CRSP Project

1. The proposed research must be innovative and feasible and must have technical merit (i.e., must pass peer review). Each proposed investigation must be clearly identified as an experiment, study, or activity, using the definitions given above in section C.4. Proper identification of proposed work is necessary, as reviewers will use different criteria for evaluating experiments, studies, and activities. Each investigation will be separately reviewed; thus, it is possible that not all investigations within a given proposal will be approved for funding.

2. Proposals may contain more than one investigation. Proposals must contain at least one research experiment or study. Proposals must not contain more than one activity. For detail on proposal preparation guidelines for experiments, studies, and activities, see Sections I and J.

3. Proposals involving pond research must also include plans for full characterization of any new research sites (RFP website: Site Descriptions). Site data should at the minimum include geographical coordinates, topographical information, baseline water quality and soils data, and a schematic of the pond layout as appropriate to the proposed investigation. Site characterization may be included as an investigation.

4. PI(s) will be responsible for interactions with the Technical Committee (TC), including but not limited to: ensuring standardization in reporting data to the database, ensuring technical progress, participating in election of TC members and serving if elected, preparing work plans for review by the TC, carrying out work plans using standardized methodologies approved by the Materials and Methods Subcommittee of the TC, and complying with program policies relating to work plan changes.

5. Proposals must identify intended beneficiaries. To this end, each proposed investigation must:
   (a) include a section on the quantifiable benefits that are anticipated (see Section J.2.e and RFP website: Quantifiable Benefits); and
   (b) identify impact indicators. Impact indicators should be quantifiable and based on the Results Framework described in the *Continuation Plan* (RFP website: Results Framework). The aim of impact indicators is to demonstrate to the funding agency how CRSP research contributes to aquaculture development. PI(s) are responsible for collecting the necessary impact information and reporting it on the required dates to the PMO (see Section J.2.g and RFP website: Impact Indicators).

6. As this Tenth Work Plan is envisioned for a 22-month horizon, proposals must contain a plan for technology adaptation and transfer (see RFP website: Technology Adaptation and Transfer). A principal objective of the CRSP is to build capacity of HC researchers and

farmers through improved understanding of aquacultural technologies (including soft technologies such as best practices and knowledge-based systems, as well as hard technologies).

7. PI(s) will be responsible for fulfilling all reporting requirements as defined by USAID and the ME. A listing of reporting requirements can be found on the RFP website (see Reporting Requirements).

8. Research priorities:

Priority Ecosystem:	Freshwater ponds/lakes and freshwater aquaculture farms; environmental research may include saline ecosystems
Priority Species:	Tilapia, polyculture species with tilapia, or native species
Target Groups:	Freshwater aquaculture farmers (small-to-medium scale, subsistence and commercial)
Key Partners:	University, governmental, and non-governmental.

H. Other Useful Information

1. **Funding History**
CRSP allocations for individual experiments and studies currently range from US$15,000 to $60,000/year, averaging approximately US$40,000/year. Activities average slightly less, at $25,000/year. The percentage of funding in each research theme and region is presented in Table 1. Funding levels depend on, among other factors, the number of investigations approved for funding. Prime site projects (see Section B)—including usually five or more investigations—are typically funded between $70,000 and $250,000 per year. Cross-cutting research projects and companion site research projects, consisting of fewer investigations, typically range from $10,000 to $65,000 per year.

2. **Allocation Period**
The Tenth Work Plan covers the period 1 July 2001 through 30 April 2003 (22 months). Funding is typically allocated for a 12-month period; however, the period may be shorter or longer depending on amount and timing of grant allocations received from USAID. The project will be incrementally funded over a 1-1/2 year period. *All* allocations are contingent on the annual funding level obtained from USAID and on performance under subcontract provisions.

3. **Termination Date**
All investigations must be fully completed and all final reports submitted by 30 April 2003.

4. **Proposal Review Process**
The CRSP ME will send proposals to external peer reviewers and internal CRSP technical reviewers. Investigations will be reviewed by individuals with expertise in the identified research theme. Proposals may contain investigations addressing different research themes; thus, the same reviewer may not review all investigations in a given proposal. Each investigation will be sent for review by at least three external reviewers. For your information, review criteria and forms are posted at the RFP website (Reviewer Evaluation Criteria). The PMO will send completed reviews to the CRSP Technical Committee (TC) co-Chairs, the TC Subcommittee on Work Plans and Budgets, and the Board of Directors (BOD). The BOD and TC will evaluate reviews along with original proposals and will make their separate recommendations to the ME. The ME will evaluate the recommendations and apply programmatic criteria for selecting investigations to be awarded under the Tenth Work Plan. The ME will notify the BOD of its decisions. The ME will then notify PIs and award subcontracts to successful proponents. A subcontract will be drawn up between Oregon State

University and the selected US University. Proposals for re-submission may be identified and the TC notified. Resubmission deadline is to be determined.

I. INSTRUCTIONS FOR PROPOSAL ORGANIZATION AND FORMAT

A checklist is provided for assembling the proposal (RFP website: Checklist for Proposal).

1. Proposals must contain the following elements (20-page limit excluding cover sheet, summary page, and excluding items listed below in 2.):

   Cover Sheet (RFP website: Cover Sheet)
   Summary Page (RFP website: Summary Page)
   Introduction (1-page limit): The text should clearly describe the overall vision of the project, and attend to the programmatic and technical considerations stated above.
   Separate descriptions for each experiment, study, and activity proposed. Limit is 4 pages per investigation (see Section J. *Instructions for Completing Descriptions of Investigations).*
   Technology Adaptation and Transfer Strategy (1-page limit) (information on website)

2. Proposals must contain the following budgetary and supporting information:

   Budget (RFP website: Budget); a separate budget per year and per investigation
   Budget justification (RFP website: Budget Justification)
   Pending funds form (RFP website; Pending Funds)
   Conflict of interest form for lead PI(s) (RFP website: Conflict of Interest)
   List of names of five reviewers (names, addresses, area of expertise) (see K.4)
   Letter(s) of Interest from partner(s)
   Statement of institutional track record and experience in the proposed HC (1-page limit)
   Plans for characterizing new pond research sites, as necessary (1-page limit)
   For new companion sites only: Site Selection Criteria response (RFP website: Site Selection Criteria Guidelines)
   Institutional supporting information:
   For all proponents:
   - Animal Use Approval (or written waivers)
   - Human Subjects Approval (or written waivers)
   - NICRA

   For proponents without existing CRSP subcontracts:
   - Institutional & Agency Certifications and Assurances (for primary US institution only) (RFP website: USAID Certifications and Assurances)

   CVs for lead PI(s) (not necessary for partners) (2-page limit per CV)

3. Proposal Format

   Use standard letter-sized paper (8.5" x 11") or A1.
   Line spacing: single space
   Minimum page margins: right - 1", left - 1", top - 1", bottom - 1".
   Minimum font size: 10 point
   Each page of the proposal subsequent to the title page must identify the principal author and title (abbreviated if necessary) and the page number; font size of header or footer may be 9 point.

   Letter quality original is preferred.

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J. Instructions for Completing the Descriptions of Investigations

1. Each investigation description must be no longer than 4 pages, including the sections described below (2 a-j) but excluding budget information and supporting information.

2. Each investigation shall contain the following sections:

   a. Title of Investigation. Below the title the following information must be provided:
      - The research theme to which the proposed investigation applies. Please use only those research themes that are mentioned in the RFP (see C.3)
      - The type of investigation (i.e., experiment, study, or activity)

      Enclose this information in parentheses, separating research theme and type of investigation by a slash. For example: Models for Heterotrophic Pond Dynamics (Aquaculture Systems Modeling/Study).
      Note: Proposals must inlcude at least one experiment or study and only one activity.

   b. PI(s)/affiliation(s)

   c. Objective(s): stated objectives should be in accordance with Results Framework for Research Areas and Specific Objectives given in the *Continuation Plan* (RFP website: Results Framework).

   d. Significance: Provide justification for conducting the proposed work, review similar and related work reported in the literature (including citations), and describe how the work relates to priorities described in this RFP and the *Continuation Plan*.

   e. Quantified Anticipated Benefits: Identify target groups and direct and indirect benefits accruing from the research and outreach work. Benefits must be quantified (RFP website: Quantifiable Benefits.)

   f. Research Design or Activity Plan
      f.1. Location of work
      f.2. Methods
         f.2.1. Pond research
            Pond facilities
            Culture period
            Stocking rate(s)
            Test species
            Nutrient inputs
            Water management
            Sampling schedule
            Statistical design, null hypothesis(ses), statistical analysis(ses)
         f.2.2. Other (non-pond) research
            Research plan and methodology

   g. Impact Indicators, specifying quantifiable measures to be used to evaluate project performance and impact. (RFP website: Impact Indicators.)

   h. Regional Integration, describing how the proposed research will integrate with regional plans. (See Section F.3 and RFP website: Regional Plans).

i. Schedule, indicating the start date and completion date of the proposed work. Final report deadline is 30 April 2003.

j. Literature Cited

K. Guidelines for Proposal Submission

1. The CRSP Program Management Office (PMO) must receive proposals by 2 April 2001.

   Sixteen (16) copies of complete proposals must be received by the PMO by close of business on 2 April 2001. Complete proposals must also be submitted on a labeled computer disk (3.5" or zip disk) in the original word processing software. Be sure to include the type of software, the version number, and the format (PC or Mac) on the labeled diskette. Faxed or emailed proposals will not be accepted.

   Mail proposals via US mail or courier to:

   Dr. Hillary S. Egna, Director
   Pond Dynamics/Aquaculture CRSP
   Oregon State University
   418 Snell Hall
   Corvallis, OR 97331-1643 USA

   Postmark date is not a criterion for acceptance consideration. Proposals not received by the PMO by 2 April 2001 are ineligible for consideration. There will be no exceptions. The PMO will confirm timely receipt of each proposal by emailing a confirmation notice to the proponent.

2. Proposals must adhere to the described format and include all requested information. Please use the *Tenth Work Plan Proposal Checklist* (RFP website: Checklist for Proposal).

3. Proposals must not exceed the stated page limit (see Section I). Proposals exceeding the page limit will not be considered and proponent notified. A corrected proposal will be accepted if deadlines are met.

4. In order to expedite the external peer review, please submit the names, addresses, and area of expertise of five potential reviewers. Also complete the Conflict of Interest form (RFP website: Conflict of Interest). External reviewers should have no obvious conflict of interest.

5. Each proposal must have been approved by the proponents' university before being considered for funding by CRSP.

6. Please direct questions regarding proposal organization and process (format, schedule, reviews) to:

   Cormac Craven, CRSP Assistant Director
   Pond Dynamics/Aquaculture CRSP
   Oregon State University
   418 Snell Hall
   Corvallis, OR 97331-1643 USA

   email: cravenc@ucs.orst.edu
   tel: 541-737-6423