EGNA

TITLE XII

COLLABORATIVE RESEARCH SUPPORT PROGRAM

POND DYNAMICS/AQUACULTURE

SEVENTH WORK PLAN

1 SEPTEMBER 1993 to 31 AUGUST 1995

REVISED 1 SEPTEMBER 1993

Pond Dynamics/Aquaculture CRSP
Program Management Office
Office of International Research and Development
Oregon State University
Snell Hall 400
Corvallis, Oregon 97331-1641, USA

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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 September 1993 through 31 August 1995. Program activities are funded in part by Grant No. DAN-4023-G-00-0031-00 from the United States Agency for International Development. Egypt activities are funded under Grant No. 263-0152-G-00-2231-00.

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Introduction

The Seventh Work Plan of the Pond Dynamics/Aquaculture CRSP was developed by the CRSP Technical Committee and describes activities to be conducted by the CRSP during the period 1 September 1993 to 31 August 1995. Egypt activities are to be conducted from 1 October 1992 to 30 September 1994.

The Seventh Work Plan will be implemented in Honduras, Rwanda, Thailand, the Philippines, Egypt, and the United States. Field studies will concentrate on the dynamics of aquaculture ponds. In addition to field activities at the research stations of the cooperating institutions, experiments with cooperating farmers are planned. Analysis and synthesis of the global data resulting from the field studies will be conducted in the United States.

The first three CRSP work plans specified identical experiments at all CRSP sites to provide a baseline for comparisons between sites. The approach was changed for the Fourth, Fifth, and Sixth Work Plans, which covered the period from September 1987 to August 1993, in that different, but related, experiments were conducted at the various sites. In this way, many more hypotheses could be tested than if only identical experiments had been conducted at all sites. The Seventh Work Plan follows the same approach as the Fourth, Fifth, and Sixth Work Plans. Different, but related, topics will be considered at each site. The particular topics to be studied at each site are based on the research needs of aquaculture in each country and the needs for more information, as identified by the CRSP Technical Committee. In addition, a global organic/inorganic fertilization experiment will be conducted at all sites.

The general goals of this work plan are:

1. To preserve the global nature of the CRSP experiments;
2. To conduct experiments to refine management practices for fertilized ponds;
3. To verify CRSP results with cooperating farmers;
4. To continue adding observations to the global CRSP database; and
5. To verify preliminary guidelines for management of fertilized ponds.

The Global Experiment

The Global Experiment will be conducted at all sites as part of the Seventh Work Plan. The Technical Committee adopted a global organic/inorganic fertilization experiment with the following elements for Honduras, Thailand, and Rwanda:

- Two precisely defined fertilization schedules and one optional schedule (to be defined by individual country projects) will be carried out at each site.
- The two precisely defined fertilization protocols are:
  1) Chicken litter-chemical fertilization schedule as defined by the PONDCLASS expert system in accordance with local conditions (Total N content in chicken litter determined by laboratory analysis.)
  2) Fixed fertilization regime of 250 kg chicken litter (DM basis)/ha/week plus urea-based chemical fertilizer so total N equals 28 kg/ha/week and sufficient P to maintain a minimum N:P ratio of 4:1

The Global Experiment in Egypt will be conducted to compare yields obtained with traditional Egyptian methods to those obtained under PD/A CRSP production practices.

Further detail of the experimental design is found in DAST Study 6, which is followed by a summary of how to use PONDCLASS.

Required Measurements

This section presents the minimum requirements for data collection at the research station. Accepted methods for data collection are presented in the Materials and Methods Handbook, which was distributed to Principal Investigators in 1992. The Standard Protocol is precisely defined in the Handbook, which should be referred to when implementing studies in which "standard protocol" are indicated. Frequencies of data collection as specified in this section are minimum frequencies. Data may be collected more frequently at the discretion of the individual projects.

The following measurements must be taken daily:

- Solar Radiation
- Wind Speed
- Air Temperature (maximum and minimum)
- Rainfall
- Evaporation
- Mortalities
- Pond Depth
- Water Inflow and Overflow

There will be at least three intensive sampling periods for each experiment: (1) during the second week; (2) midway through the experiment; and (3) during the final week. Whole column samples collected at mid-morning should be used unless specified otherwise. The variables to be observed are:

- Total Kjeldahl Nitrogen
- Ammonia Nitrogen
- Total Phosphorous
- Secchi Disk Visibility
- Chlorophyll *a*
- Dark Bottle Respiration
- Total Suspended Solids
- Total Volatile Solids
- Total Alkalinity (3 depths: top, middle, bottom)
- Primary Productivity

Diel studies will be conducted simultaneously with the intensive sampling measurements in order to measure spatial and temporal fluctuations within a pond. Samples for diel studies will be collected at dawn, 1000, 1400, 1600, 1800, and 2300 hours, and at dawn the next day at a minimum of two depths, but preferably at three depths. The exact time of sample collection in the diel studies should be recorded. The three sampling depths will be

25 cm below the water surface, mid-depth, and 25 cm above the pond bottom. The parameters to be measured during the diel studies are:

- Dissolved Oxygen
- Temperature
- pH
- Wind (cumulative between sampling times)
- Solar Radiation (cumulative between sampling times)

Information about the fish and shrimp used in the experiments should be recorded as follows:

– Stocking
  - Total Number
  - Total Biomass
  - Individual Weights (of 10% sample)
  - Individual Lengths (of 10% sample)

– Monthly Sampling
  - Total Number in Sample
  - Total Biomass of Sample
  - Individual Weights
  - Individual Lengths
  - Reproduction Weight

– Harvest
  - Total Number of Stocked Fish Remaining
  - Total Biomass of Stocked Fish
  - Individual Weights (10% sample of stocked fish)
  - Individual Lengths (10% sample of stocked fish)
  - Total Number of Recruits
  - Total Biomass of Recruits

The following pond soil characteristics are to be determined at the beginning and end of each experiment:

- pH
- Phosphorus
- Organic Matter
- Total Nitrogen
- Cation Exchange Capacity
- Metals - Aluminum, Iron, Zinc (only when the pond is first used)
- Lime Requirement
- Exchangeable Hydrogen
- Base Saturation

Pond morphology is to be measured when the ponds are first constructed and whenever pond morphology is altered significantly. Measurements to be taken are:

- Surface Area (at 10 cm depth contours)
- Volume (at 10 cm depth contours)
- Drawing, top view, with scale

The composition of lime, inorganic, and organic fertilizers is to be determined when supplies are delivered and, for organic materials, just before they are totally used up, but not less frequently than once a month. Characteristics to be determined are:

- Percent Dry Matter
- Nitrogen
- Phosphorus
- Chemical Oxygen Demand
- Lime Neutralization Value (for lime only)

The quantities of lime and other amendments must be carefully recorded whenever they are added to the ponds.

Reference ponds are to be established and operated at each station starting the second year of this work plan.

The standard sampling schedule for on-farm studies is as follows:

Initial sampling:
- Water: alkalinity, hardness, conductivity
- Soil: pH, color/texture (e.g., red, brown, sandy, loamy, clayey, peat)
- Fish: total weight and number

Monthly sampling:
- Water: alkalinity, pH, DO, check/calibrate max-min water thermometer, time of sampling
- Fish: total weight and count of a sample of at least 30 animals

Weekly sampling (by farmer):
- Max-min water temperature, Secchi disk visibility, water color by comparator chart, type and quantity of pond inputs.

"When needed":
- Fish mortality (number, length)
- Comments
- Water input: add water when level drops 10 cm below full-pool.

Harvest:
- Population total weight and count, separated by sex.

Optional Measurements

CRSP projects may collect any data, in addition to those data specified under Required Measurements, which they deem appropriate for a particular study. The methods specified in the Materials and Methods Handbook should be used. If a method for a particular parameter is not specified in the Materials and Methods Handbook a method from Standard Methods (APHA et al., 1985) should be used whenever possible and the Materials and Methods Subcommittee of the CRSP Technical Committee should be informed. If problems are encountered while using the accepted method for a particular application, the Materials and Methods Subcommittee should be contacted. If optional measurements are made and researchers wish to have the data included in the data templates, the Data Base Manager (at the University of Hawaii, Hilo) should be contacted.

Data Submission

All data should be submitted to the CRSP Database Manager on either Lotus 1-2-3® or Microsoft Excel® worksheets, following the formats and procedures in the most recent CRSP Instructions for Data Entry. Please contact the Data Base Manager with questions regarding verification of data.

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Report Submission

A technical report for each experiment (each study) is due six months after the experiment ends. Technical reports should be submitted in one of the following formats:

a) As a Research Report, with the understanding that the manuscript will be reviewed, edited, and printed as a short (4-8 pages) report; or

b) As a Notice of Publication under the Research Reports series. Notices of Publication are reserved for those articles that have been or will be published in a refereed journal. Authors who wish to use this format for technical reports should submit a copy of a manuscript which is "under review" by a journal, together with a cover letter. It is recommended that authors contact the CRSP Director to discuss whether this is an option.

In addition, researchers will submit for publication in the Annual Report a technical report for each experiment completed during the reporting period from 1 September to 31 August of each year. Should difficulties in completing the Work Plan on schedule arise, please advise the Director so that an alternate reporting format can be arranged.

Honduras Project

Cooperating Institutions:

Auburn University
Dr. Claude E. Boyd
Dr. Bryan L. Duncan
Dr. David R. Teichert-Coddington

General Directorate of Fisheries and Aquaculture, Ministry of Natural Resources
Ing. Mauro Pino Merren

Introduction:

The studies in Honduras will concentrate on the brackishwater environment in Choluteca, southern Honduras, while maintaining a secondary freshwater site at the El Carao National Aquaculture Research Center. The primary study area will encompass selected shrimp farms and the estuaries which supply water to, and receive effluents from the farms. Little is known about the water quality of this environment. A baseline of information on shrimp farms, estuaries and their interactions will be established. Specific farm resource management issues will also be addressed by means of replicated pond studies.

Interaction between shrimp farming and estuarine ecology are important issues for the nascent shrimp industry in Honduras and neighboring Central American countries. Experience in southeast Asia and Indonesia demonstrates that shrimp farming can become untenable if farm discharge exceeds the carrying capacity of the estuaries. Incidence of disease increases and production falls as the water quality deteriorates. Shrimp farming regions of Honduras and Nicaragua share the Gulf of Fonseca as their water reservoir. The gulf is shallow and is reported to exchange its total volume of water with the Pacific Ocean about once every seven years. Therefore, the potential for deterioration of gulf water quality also exists. Every shrimp farming area is different because of estuarine size, estuarine exchange rates with the ocean or bays, freshwater inflow, and the amount and type of land suitable for farming in a particular area. Shrimp farming development must therefore be accompanied by objective studies that make information available on environmental impact of farming for self or governmental regulation of the industry. The industry can also be encouraged to practice management techniques that will minimize deterioration of its water supplies.

Honduran shrimp farms are located on several different estuaries of the Gulf of Fonseca where water quality varies widely in a relatively small geographic area. Estuaries comprising the confluence of major rivers (Choluteca and Negro) and the Gulf are muddy and carry city and agricultural effluents. Salinity fluctuates widely during the year, dropping to 5 ppt or less during the rainy season and rising in excess of 45 ppt during the dry season. The appearance of the water also changes with seasonal rains. Estuaries formed from inlets of the Gulf are relatively clear and apparently non-polluted, and fluctuate much less in salinity and apparent water quality than the riverine estuaries. Study of the interaction between shrimp farming, estuarine water quality and climate would yield practical results for the shrimp producer and environmental manager, and information for the systems dynamics modeler. This information will not be restricted in use to Honduras, but will have application worldwide where shrimp are being cultured.

Replicated pond studies will focus on methods for increasing production efficiency in a sustainable fashion. Efficient use of shrimp post-larvae, feeds, fertilizers and water directly impact environmental quality and farm profitability. Over feeding and fertilizing results in wasteful nutrient discharges. Higher stocking densities imply greater use of larvae which may be captured from the estuaries, greater water exchange, higher feeding rates per pond area, and greater nutrient discharge. Despite the economic importance of shrimp farming, there is a paucity of scientific information on brackishwater pond dynamics and its practical use in farm management.

Work at the El Carao fresh water site will tie up some loose ends from prior studies on combinations of organic and inorganic fertilizers, and provide new data on minimal use of water exchange as a method for increasing yields. Use of supplemental nitrogen fertilization has increased fish yields, but it has also decreased water quality in relation to pH and blue-green algal blooms. More judicious use of nitrogen is warranted. The work plan will concentrate on reducing N input based on total ammonia levels, and will use a different form of nitrogen in order to reduce pH and ammonia levels. The work will also test PONDCLASS, a computerized management tool developed by the CRSP, for optimizing fish yields.

Honduras Study 1: Estuarine Water Quality

Objectives: Establish a baseline of information on selected chemical, biological, and physical characteristics of water at points along major estuaries that supply water and receive effluents from shrimp farms.

Significance: No repeated measurements of chemical, physical and biological data of estuarine water are known for southern Honduras. This study will establish a baseline of information against which environmental impact of shrimp farming on the estuaries can be measured. It will provide a means by which estuaries can be stratified by type, level of pollution, and potential for degradation. These data will be useful in discovering the causes of yield differences among farms in the area.

Experimental Design:

1) Choose 6 to 8 locations (shrimp farms) on different estuaries or different sections of a major estuary for sampling. A sample from the Choluteca river before it receives effluent from the city will be used as a reference point. Other reference points upstream of shrimp farm activity will be sought.

2) Sample estuarine influent water at the pumping station at about the middle of the daily pumping cycle (high tide).

3) Periodic sampling of water at the pumping station through a pumping cycle (pumping is not continuous and corresponds with tidal cycles) to establish variation of influent water with tidal flow.

4) All stations will be sampled once a week during a calendar year. Sampling will continue after the first year, but at a reduced frequency.

Variables Measured: Total settleable solids; COD; total nitrogen; total ammonia nitrogen; total phosphorus; chlorophyll *a*; salinity; temperature; dissolved oxygen. River flow, rainfall and evaporation data will be obtained from the Directorate of Hydrology, Ministry of Natural Resources, Honduras.

Statistical Methods: Stratification of estuaries by type; monthly averages by location and by estuarine type.

Schedule: Data collection, March 1993 - 1995

Honduras Study 2: Farm Nutrient Budgets

Objective: Quantify nutrient flow into and out of farms located on different estuarine types.

Significance: Little is known about effects of shrimp farming on enrichment of estuarine water. Large quantities of nutrient and organic rich loading from ponds could eventually lower estuarine water quality. Quantification of nutrient flows through ponds would allow for estimates of pollution intensity of farms per area of pond and biomass of shrimp produced.

Experimental Design:

1) Choose 4 farms on which nutrient flow into and out of the farms will be measured during a calendar year. Two farms located on each of the major estuarine types will be sampled. Feeds and fertilizers applied to ponds, and harvested shrimp will be included in the nutrient flow.

2) Water in the supply and drainage canals will be sampled. Sampling points along the outfall of canals will be established to ensure that a good nutrient profile is being captured. Subsamples will be analyzed separately to establish variation encountered along canals.

3) Farms will be sampled every two weeks.

4) If time and labor permit, water entering and leaving individual ponds will be sampled, giving special attention to discharge during harvest.

Variables Measured: Total settleable solids; COD; total nitrogen; total ammonia nitrogen; total phosphorus; chlorophyll *a*; salinity; temperature.

Statistical Methods: Nutrient load and discharge per unit of water, area of pond, and biomass of shrimp; stratification by estuarine type; ANOVA.

Schedule: Data collection, April 1993 - March 1994

Honduras Study 3: Replicated Studies in Small (≤1-ha) Ponds

Honduras Study 3A. Reduction of feed input by inorganic fertilization.

Objective: Compare yields of *P. vannamei,* primary productivity and water quality in ponds receiving feed or combinations of feed and inorganic fertilizers.

Significance: Preliminary data indicate that regardless of growing season, shrimp yields do not increase at higher diet protein level, at stocking densities between 5 and $10/m^2$. There are indications that lower levels of feeding could be employed without impacting shrimp yields, especially during the "poor" growing season when shrimp appear to consume less.

The use of inorganic fertilizers in trials carried out on Choluteca shrimp farms has resulted in greater primary productivity, but not in greater shrimp yields. However, the trials were not run during the "good" growing season when shrimp might be better able to take advantage of greater primary production. The substitution of organic fertilizers for feed during the first eight weeks of growth has resulted in improved profitability. However, organic fertilization is not now widely practiced, because of fear that the exported product will be contaminated. It may be that inorganic fertilizers may be similarly substituted for feed.

Null Hypothesis: Primary productivity and shrimp yields will be independent of feeding rate or and supplemental inorganic fertilization.

Experimental Design: Ponds will be stocked with *P. vannamei* juveniles at $7.5/m^2$. Four treatments will be tested with each treatment replicated three times:

1) Feed offered at the normal rate;
2) Feed offered at half the normal rate;
3) Feed offered at half the normal rate, and inorganic fertilization;
4) Inorganic fertilization during the first eight weeks followed by the normal feeding rate.

The fertilization program will follow those used in the CRSP freshwater trials. Nitrogen and phosphorus in the forms of urea and ammonium phosphate will be added weekly at 20 and 4 kg/ha, respectively. Rates will be reduced if total ammonia concentrations are higher than 0.25 mg/l two weeks in a row. This experiment will be repeated during both the good and bad growing seasons of the year.

Variables Measured: Shrimp production; CRSP water quality protocol.

Statistical Methods: ANOVA.

Schedule: Data collection, July 1993 - March 1994.

Honduras Study 3B. Influence of frequency and quantity of water exchange on water quality and shrimp production.

Objective: Evaluate the effect of water exchange frequency and quantity on water quality and shrimp production.

Significance: Exchange in shrimp ponds is probably practiced universally regardless of management system. In semi-intensive culture, water is exchanged primarily to correct low early morning oxygen concentrations. It is also thought to discharge metabolites which hinder shrimp growth. Water exchange is undoubtedly beneficial, but not enough is known to optimize quantity and frequency of water exchange. Excessive water exchange wastes fuel, and contributes to deterioration of pumps and sedimentation of water supply canals and ponds.

Null Hypothesis: Shrimp yields will be independent of frequency and quantity of water exchange.

Experimental Design: A completely randomized block design will be used to test two frequencies and two quantities of water exchange: daily at 10% of volume; daily at 5% of volume; twice a week at 20% of volume; twice a week at 10% of pond volume. Stocking rate will be $10/m^2$. The experiment will be repeated during good and poor growing periods.

Sampling Schedule: Shrimp growth, salinity, Secchi disk visibility and chlorophyll *a*, weekly; early morning dissolved oxygen and temperature, daily; CRSP water quality protocol three times during the cycle.

Statistical Methods: ANOVA with pre-planned comparisons between quantity within each level of frequency, and between levels of frequency (daily vs. twice a week).

Schedule: Data collection, June 1994 - March 1995.

Honduras Study 4: El Carao - Freshwater Site

Honduras Study 4A. Regulation of input and type of nitrogen used for supplementation of chicken litter (Global Study).

Objectives:

1) Test effect on primary production and fish yields of regulating urea input by total ammonia measurements;

2) test effect on water quality, primary production, and fish yields of supplementing organic fertilization with nitrate-nitrogen compared with ammonia-nitrogen;

3) test the utility of PONDCLASS as a pond management tool.

Significance: Use of supplemental urea fertilization has resulted in high pH (> 10), sometimes high total ammonia concentrations, and frequent blue-green algal blooms. Fish production appears to be adversely affected at high nitrogen rates. It is suspected that the high pH's have favored blue-green algal surface scums possibly by lowering dissolved carbon levels in ponds, and that the combination of relatively high ammonia concentrations and noxious products of blue-green algae may result in lower fish production.

Previous studies have consistently demonstrated that total ammonia greater than 0.15 mg/l has not resulted in greater primary production. Regulation of nitrogen input according to this concentration should result in lower nitrogen inputs, lower ammonia levels and possibly lower pH's. It is also surmised that ammonia and pH levels could be reduced through use of nitrate instead of ammonia-forming fertilizers.

Null Hypotheses: Fish yields will be independent of management regime or type of nitrogen fertilizer used; pH and ammonia concentrations will not be different between treatments 1 and 2 because of type of N fertilizer used.

Experimental Design: A 1-factor experiment consisting of a completely randomized design with four treatments will be conducted. Treatments will be replicated three times. Ponds will be stocked with *Oreochromis niloticus* at 2/m² and guapote tigre at 500/ha. Treatments are as follows:

1) Weekly applications of chicken litter at 250 kg/ha, urea to maintain weekly total N input of about 28 kg/ha, and ammonium phosphate to maintain total P input in relation to N of 1:4.

2) Equal to treatment 1 except that KNO_3 instead of urea will be used to supply N.

3) Equal to treatment 1 except that urea inputs will be adjusted to maintain total ammonia levels of about 0.15 mg/l. Urea will be reduced by 25% when total ammonia rises above 0.18 mg/l and increased again when levels drop to 0.12 mg/l.

4) Use quantities of chicken litter, N (urea) and P as recommended by PONDCLASS.

Pond Facilities: El Carao, twelve ponds, 0.90 m deep, each 0.1 ha.

Culture Period: 150 days.

Sampling Schedule: early morning DO, total ammonia, and chlorophyll *a* once a week; primary productivity, pH, total P, total hardness, total alkalinity, and total N three times during the study.

Statistical Methods: ANOVA.

Schedule: July 1993 - October 1993.

Honduras Study 4B. Stocking rate of *Colossoma macropomum* in polyculture with tilapia.

Objectives: To determine the relative growth potential of tambaquí (*Colossoma macropomum*) in polyculture with tilapia, and the optimum stocking rate for tambaquí in this polyculture.

Significance: Tambaquí and tilapia are both capable of fast growth. Tambaquí are apparently capable of faster weight gain than tilapia, especially at mean weights over 400 grams. Tambaquí require a feed to grow well, whereas tilapia will grow well on natural pond food as well as feed. It could be that a polyculture of the two fish under fed conditions is more efficient than a monoculture of either.

Null Hypothesis: Fish yields will be unaffected by proportion of stocked species.

Experimental Design: Completely randomized design. Four treatments by three replicates/treatment:

1. Tilapia (100%)
2. Tilapia (75%) plus tambaquí (25%)
3. Tilapia (25%) plus tambaquí (75%)
4. Tambaquí (100%)

Pond Facilities: El Carao, twelve ponds, 0.90 m deep, each 0.1 ha.

Culture period: 180 days.

Fish Stocking Rate: Male *Oreochromis niloticus* and tambaquí fingerlings will be stocked in the proportions indicated in the experimental design such that the total number of fish stocked is 30,000/ha; in addition, guapote tigre (*Cichlasoma managuense*) fingerlings will be stocked at 750/ha.

Nutrient Inputs: Chicken litter (500 kg TS/ha/wk) and urea to bring total N input to 30 kg/ha, and feed (28% protein) at 1% of fish biomass in treatment 4, during the first month. Thereafter, fertilization will be stopped, and feeding rate increased to 3% of fish biomass in treatment 4. When daily early morning temperatures drop below 23°C, then the feeding rate will be reduced to 2%. Feed will be offered in two meals, six days a week.

Water Management: Replace evaporation and seepage; during the last month of growth, 25% of pond volume will be exchanged once a week.

Sampling Schedule: Standard protocol (water chemistry sampled three times during study).

Data Analyses: ANOVA, orthogonal comparisons.

Schedule: October 1993 - March 1994.

Honduras Study 4C. Effectiveness of water exchange at maintaining minimum oxygen levels.

Objectives: Test the effectiveness of water exchange for maintaining a minimum oxygen level, and the consequences of the manipulation of water exchange volume per exchange episode on water exchange efficiency.

Significance: In an earlier study, fish yields were significantly higher in ponds aerated to maintain DO above 10% of saturation. However, aerators are expensive and require a regular source of electricity. Water exchange may be used to accomplish the same goal as aerators at moderate fish stocking densities. Water is a valuable resource that must be used wisely. Therefore, research on how to efficiently exchange water needs to be accomplished. Greater volume of exchange per exchange episode may be more efficient than more frequent episodes of smaller volumes of water. This study will indicate the usefulness of water exchange for maintaining a minimum DO level, and whether exchange efficiency can be improved by manipulating volume of exchange per exchange episode.

Null Hypotheses: Water exchange will not be an effective means for maintaining minimum DO levels; there will be no difference in total water exchanged or DO concentrations because of the percentage of pond water exchanged per exchange episode.

Experimental Design: A completely randomized design in 2 X 2-factorial arrangement will be used. Treatments will be replicated three times. Ponds will be stocked with *Oreochromis niloticus* at 3 or 6/m^2 and guapote tigre at 500 or 1000/ha. If the early morning DO falls below 15% of saturation, then water will be exchanged at 10% or 40% of pond volume at each stocking density.

Pond Facilities: El Carao, twelve ponds, 0.90 m deep, each 0.1 ha.

Culture Period: 150 days.

Nutrient Input: During the first month, ponds will be fertilized with chicken litter at 500 kg/ha, urea to maintain weekly total N input of about 30 kg/ha, and ammonium phosphate to maintain total P input in relation to N of 1:4. They will also be fed with a complete diet at 1% of body weight. Thereafter, fertilization will be stopped and fish will be fed a commercial diet consisting of 28% protein at 3% of body weight, six days a week. The daily diet will be divided into three meals. Fish biomass will be estimated every two weeks.

Sampling Schedule: Daily early morning DO; weekly chlorophyll *a*; primary productivity, pH, total P, total hardness, total alkalinity, and total N three times during the study.

Statistical Methods: ANOVA.

Schedule: May 1994 - October 1994.

Rwanda Project

Cooperating Institutions:

National University of Rwanda
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Mr. Anaclet Gatera

Oregon State University
Mr. Wayne Seim
Dr. Richard Tubb

Auburn University
Dr. Thomas Popma
Ms. Joyce R. Newman

University of Arkansas at Pine Bluff
Dr. Carole Engle

Introduction:

Rwandan fish ponds are located in highland equatorial Africa in the cooler portion of the temperature range suitable for tilapia culture. The Rwanda project over the next two years will work toward a sufficient understanding of pond dynamics to develop recommendations of elevation-dependent management strategies for tilapia production. Recommendations based on progress thus far, and on the next series of activities, can be expected to improve fish production and economic gain compared to existing practices.

Proposed research activities under the Seventh Work Plan are based on the following considerations:

1. Fish stocking density for maximum production varies with elevation-related factors including temperature and system productivity. Higher fish stocking densities may be appropriate at higher elevations if reduced fish growth results more from depressed food consumption than from lower system productivity. But if reduced primary productivity is the principal constraint to fish growth at high elevations, lower stocking rates and/or supplementary feeding may be most effective at enhancing fish production. These questions can be tested most effectively with a combination of pond and laboratory experiments.

2. Additional data on the influence of elevation dependent factors on tilapia production have been collected over several years by Rwandan extension agents, but have never been transcribed to a user-friendly format for analysis. Such an exercise may permit insight into the relationships between fish production, elevation, soil characteristics, and the response of producers to elevation-related conditions.

3. The appropriateness of suggested elevation-dependent management strategies must, in the end, be evaluated by collecting biological and economic data from farmer-owned ponds. This approach integrates both cultural and biological factors leading to correct choices to maximize benefits.

Rwanda Study 1: Optimum Stocking Density in Relation to Elevation

Objective: Determine the most appropriate stocking density for male Nile tilapia in enriched ponds located in distinct elevation zones.

Significance: This activity was designed to "tie up previous and current research" and "refine pond management guidelines" (Topics 1 and 4 of suggested subject matter for the Seventh Work Plan). The results will be of value in development of the Expert System.

As water temperature decreases, the physiological capability of tilapia to consume available food likely decreases more rapidly than does the capacity of the pond to produce natural food organisms. Consequently, optimum fish stocking density for a given nutrient input regime may vary as a function of elevation. Previous research (the Sixth Work Plan) evaluated enrichment and feeding regimes at different elevations when fish stocking densities were held constant at 1 fish per m^2.

Null Hypotheses: Pond dynamics and tilapia growth and production are not a function of elevation in Rwanda; Optimum stocking density does not change with elevation.

Experimental Design: Three elevation ranges (<1600m, 1800±100m, and 2100±100m), three fish stocking densities, with each treatment replicated in five farmer-managed ponds. In addition, nine ponds will be added at Rwasave Station as a control on on-farm procedures and adherence to experimental protocols.

Pond Facilities: Forty-five farmer-owned ponds, 15 at each of three elevation ranges plus nine CRSP ponds at Rwasave Station.

Culture Period: Until fish reach an average weight of 150g but not less than 5 nor more than 7 months.

Fish Stocking Rate: Juvenile male *Oreochromis niloticus* at 1, 2 or 3 per m^2.

Nutrient Inputs: 250 kg/ha/wk dry weight basis of a fresh, locally available grass plus urea and TSP so that inorganic N and P equals 6 and 1.5 kg/ha/wk, respectively. Fertilization will be suspended during weeks in which Secchi disk readings the previous week were less than 20 cm.

Water Management: Replace evaporation and seepage losses weekly.

Sampling Schedule: Standard protocol at Rwasave Station. Sampling protocol for on-farm studies will include weekly measurements of Secchi disk visibility in all ponds and max-min water temperature in at least one pond per elevation range; monthly measurements include chlorophyll *a*, pH, alkalinity, hardness, and fish weight.

Statistical Methods: ANOVA, regression analysis.

Schedule: April 1994 - November 1994.

Rwanda Study 2: Fish Production Relationships Derived from Existing Farmer-Generated Data

Objective: Tabulate and analyze production data available from private fish farmers in Rwanda.

Significance: This PD/A CRSP task would be an effective and efficient collaborative effort. Data analysis conforms to Topic 3 of suggested activities for The Seventh Work Plan.

The Rwandan National Fish Culture Extension Service has collected farmer-generated data since 1984. Computer-assisted analysis of this information from more than 1000 fish production cycles, as a tool to evaluating pond management practices, was facilitated by the development of a spreadsheet format through the USAID-funded Natural Resources Management Project. Unfortunately, that project lacks required funds for data entry and analysis.

Experimental Design: Farmer-generated data sheets recorded by extension agents from the Rwanda National Fish Culture Service since 1984 will be transcribed to an existing spreadsheet format. Data will be analyzed to determine relationships between the following variables: fish production, stocking rate, fish population structure, fish species composition, elevation, nutrient inputs, culture period, producer characteristics and other pertinent variables. Data will be entered at Auburn University and the Rwasave Fish Culture Station, and analyzed at OSU and Auburn University.

Pond Facilities: About 1,000 records from Rwandan rural ponds.

Statistical Methods: ANOVA, regression analysis.

Schedule: June 1993 - May 1995.

Rwanda Study 3: Training Workshops for Rwandan Fish Culture Extension Agents and Producers

Objective: Extend aquacultural technology, especially that generated by the PD/A CRSP, to Rwandan extension agents and producer-leaders. This planned activity conforms to Topic 2 of suggested subject matter for the Seventh Work Plan to "train and develop training materials for extension agents."

Significance: The technological constraint to increased fish production in Rwanda is no longer the lack of proven production packages but rather the delayed dissemination of this information to producers. Production practices evaluated by PD/A CRSP and other aquacultural researchers worldwide can be effectively transmitted by PD/A CRSP personnel to extensionists and selected influential fish farmers by means of periodic workshops.

Experimental Design: Three-to-five-day workshops will be conducted at Rwasave Station and at rural pond sites. Participants will include extension agents from Rwanda and from neighboring countries, influential farmers, and program administrators. Training will emphasize proven fish culture practices, extension methodology, and economic analysis. Workshops will be offered twice annually. Number of participants at each workshop will be about 10 to 20.

Pond Facilities: Rwasave, Kigembe, and private ponds and facilities, as needed.

Schedule: September 1993 - August 1995.

Rwanda Study 4: Growth, Consumption, and Feed Conversion Efficiency of Tilapia in Aquaria in Relation to Temperature Regime

Objective: Determine in aquaria the extent to which appetite, growth, and feed conversion efficiency are affected by temperature when feed is provided frequently and at varying rates. This study conforms to Topic 1 (to tie up previous and current research) of suggested topics for the Seventh Work Plan.

Significance: On-going and planned field experiments in Rwanda focus on the effects of elevation on tilapia production. Controlled experiments will help isolate effects of temperature from other environmental variables linked to elevation (soil characteristics, solar radiation, etc.). The University of Arkansas at Pine Bluff conducted controlled experiments in aquaria on the effects of temperature and diel fluctuations in temperature on appetite, growth, and feed conversion efficiency of Nile tilapia when feed was offered to satiation once or twice daily. A more common condition, however, in enriched fish ponds is that food is available constantly and at levels below satiation.

Null Hypotheses: Appetite, growth, and feed conversion efficiency are not affected by temperature; appetite, growth, and feed conversion efficiency are not affected by temperature fluctuations.

Experimental Design: Three feeding rates; four temperature regimes, two constant (22 and 26° C), and two with diurnal fluctuations (22±4 and 26±4° C); three replicates per treatment.

Facilities: Thirty-six 40-liter aerated aquaria at Rwasave Station and/or Auburn University.

Culture Period: 3 to 4 weeks plus 1 week acclimation

Stocking Rate: 10 to 12 juvenile male *Oreochromis niloticus* per aquarium.

Nutrient Inputs: Floating fish ration with crude protein content of approximately 36%, fed to satiation, 60% satiation, and 30% satiation. To determine satiation (feed weight expressed as percent of fish weight), feed amount consumed in 20 minutes will be measured at 2-hour intervals from 8AM to 6PM on day one and weekly thereafter. Fish at restricted rations will start one day later than fish fed to satiation, to allow calculation of rations. Dead fish will not be replaced, but feeding rates will be adjusted accordingly.

Water Management: Immersion heaters will be used to maintain well water at desired temperature regimes. Detrital material will be siphoned daily from all aquaria. Water inflow will be adjusted so that minimum DO exceeds 3 mg/L and unionized ammonia does not exceed 0.06 mg/L.

Sampling Schedule: All fish will be marked and weighed individually at the beginning of the study and weekly thereafter. Temperature will be measured daily at two-hour intervals during daylight hours. At least twice during the experiment, temperature in each treatment will be continually recorded for a 24-hour period. Ammonia and pH will be measured twice weekly.

Statistical Methods: Factorial ANOVA, regression analysis.

Schedule: April - June 1993.

Rwanda Study 5: Growth of Tilapia in Relation to Temperature Regime in Aquaria Receiving Plankton-Rich Water

Objective: Quantify the effects of two naturally fluctuating temperature regimes on a naturally occurring plankton community from a single pond.

Significance: Refer to Activity 4 for general significance. This work provides a tighter link between the field and controlled studies on temperature, and may help explain the relative importance of temperature versus other environmental variables associated with elevation in Rwanda.

Null Hypothesis: Water temperature has no effect on growth and production of tilapia when plankton is the primary food source.

Experimental Design: One planktonic food source; two fluctuating temperature regimes (approximately 22 ± 3° C and 26 ± 3° C); 3 replicates per treatment. This experiment will be similar to Activity 6 except the food source will be plankton-rich water pumped from a fertilized fish pond, and only variable temperature regimes will be tested. The temperature of the water will vary naturally from approximately 19° C in the morning to approximately 25° C in the afternoon. The warmer temperature regime will be accomplished with immersion heaters in each aquaria.

Facilities: Thirty-six 40-liter aerated aquaria at Rwasave Station and/or Auburn University.

Culture Period: 4 to 5 weeks plus 1 week acclimation.

Stocking Rate: 10 to 12 juvenile male *Oreochromis niloticus* per aquarium.

Nutrient Inputs: The pond will be fertilized with chicken litter at 250 kg/ha for two weeks and weekly with urea and TSP at a rate of 16 and 4 kg/ha N and P, respectively. Plankton-rich water from pond will be pumped to all aquaria.

Water Management: Pond water will be pumped continually to the aquaria, allowing aquaria water temperature to fluctuate with diel changes in pond temperature. Water exchange rate will be about 250 ml/min or sufficient to allow a 4° C differential between the two temperature regimes. Immersion heaters will be used to maintain one set of aquaria approximately 4° C warmer than pond water. Detrital material will be siphoned daily from all aquaria.

Sampling Schedule: All fish will be marked and weighed individually at the beginning and end of the study and weekly thereafter. Temperature will be measured daily at two-hour intervals during daylight hours, and for each treatment, continually recorded over 24 hours at least twice. Dissolved oxygen will be measured daily. Ammonia and pH will be measured twice weekly.

Statistical Methods: T-test, regression.

Schedule: July - October 1994.

Rwanda Study 6: Economic Evaluation of Elevation-Dependent Management Strategies in Privately Managed Ponds

Objective: Evaluate the social, economic, and technical validity of applying CRSP research results to fish farming in Rwanda and other countries in which cash availability is a constraint.

Significance: Pond management strategies that provide maximum benefit to producers are influenced by multiple variables, but in Rwanda two of the most influential are elevation and availability of cash to purchase inputs. In this study two 'best estimates' of optimum pond management strategies will be designed for each of three elevation zones; one strategy will be designed on the assumption that cash inputs are not feasible, while the other strategy assumes that the farmer is capable and willing to invest cash. Each practice will be field tested by participating farmers.

Null Hypotheses: Return to cash and non-cash strategies are similar. Economic return for optimum strategies is not different at the elevations tested.

Experimental Design: Variables are: 3 elevation ranges (see Activity 1); 2 management strategies (one requiring no cash, and a second in which cash is available), with each treatment replicated in 5 rural ponds. In addition, the mid-elevation management packages will also be replicated in triplicate at Rwasave Station. Choice of the selected 'optimum' management strategies for non-cash input regime will be based on maximum economic returns to land and labor; optimum management strategies for cash input regime will be based on maximum absolute and percent economic return to cash inputs. Logistical constraints, availability of inputs, and risk are also criteria in selection of management practices.

Pond Facilities: 30 farmer-owned ponds, plus 6 at Rwasave Station.

Culture Period: Until fish reach an average weight of 150g but not less than 5 nor more than 7 months.

Stocking Density: Variable, see Experimental Design.

Nutrient Inputs: Variable, see Experimental Design.

Water Management: Variable, see Experimental Design.

Sampling Schedule: Same as Study 1.

Statistical Methods: T-tests or non-parametric alternatives.

Schedule: December 1994 - July 1995.

Rwanda Study 7: Risk Analysis of Fish Production in Rwanda

Objective: Undertake an economic risk analysis, incorporating the observed variability in key production parameters encountered during a previous survey of private producers.

Significance: Fish culture in Rwanda is an activity undertaken within a subsistence agricultural economy. Previous work determined that much of the fish produced is consumed by the household, but nearly half is sold to generate cash income. Previous studies have also assessed economic trade-offs between producing fish and other crops. None of these studies included risk in the analyses. Yet subsistence farmers, more so than other farmers, are less able to make production decisions that entail high levels of risk because the result of an adverse outcome may be extreme food shortages or even starvation for the household. Economic analysis that incorporates risk elements may be far different from analyses that lack risk. Enterprise budgets are derived from average values for inputs and outputs of an enterprise, but risk analysis incorporates the observed variability in these parameters, thus permitting description of the probability of economic results above or below the average. Risk analysis may result in the most valid and accurate results in a subsistence context.

Experimental Design: The database generated from Rwanda Study 5 of the Sixth Work Plan will be used for this analysis. It consists of survey data from 250 Rwandan fish farmers, both individuals and groups. The mathematical programming model currently under development for the previous work will be expanded into a risk programming model that substitutes distributions of possible values of key parameters rather than a single point estimate of the mean for a given parameter. The resulting outcomes of the analysis will be the selection of optimal product mixes for varying levels of risk aversion for production of staple crops and of cash crops. Analyses will be performed primarily on the campus of the University of Arkansas at Pine Bluff under the direction of Dr. C. Engle.

Schedule: October 1993 - May 1995.

Thailand Project

Cooperating Institutions:

University of Michigan
Dr. James Diana
Dr. C. Kwei Lin

University of Hawaii
Dr. James Szyper
Dr. Kevin Hopkins

Royal Thai Department of Fisheries, Bangkok
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Asian Institute of Technology, Bangkok
Dr. Peter Edwards

Introduction:

The proposed studies in Thailand will concentrate in two major lines: outreach activities or off site testing of CRSP results, and enhanced production systems for tilapia using either supplemental feeding or polyculture. These studies will be conducted in at least four sites: Asian Institute of Technology, Bang Sai Fisheries Station, Udorn Fisheries Station, and the Freshwater Aquaculture Center, Central Luzon State University, Philippines. Thus the Thailand studies will attempt to become more regionally oriented and include sites in other areas of Asia.

A major change of direction is being implemented in the Asian research group, which now comprises the University of Hawaii and the University of Michigan. This change is a logical extension of the earlier work and of our proposal for on-farm and regional studies. The line of research on semi-intensive culture of tilapia is becoming more refined, while many other types of tilapia culture systems are much less known. One goal of this redirection is to apply our work on pond dynamics to these lesser known types of culture. The first application has already begun with experiments in the Philippines, and this work is proposed to continue for the current work plan. The second new site will be several fishery stations in the northeast area of Thailand. We will initiate studies in conjunction with the AIT outreach project, using sites of mutual interest and systems of research identified partly by their outreach efforts. This effort is currently focused in Udorn but also includes sites in other parts of Thailand as well as Cambodia and Laos. The future of this interaction may allow the Asia CRSP group to become even more regional in its approach.

The studies included under this new direction include three proposed mainly by University of Michigan (studies 1, 2, and 4) and three proposed by University of Hawaii (studies 7, 9, and 10). We hope to conduct these studies on locations remote from Bangkok, but are prepared to do them in the Bangkok area if the proposed ponds or other facilities are unavailable. The studies in Thailand will emphasize production systems for low intensity, rainfed aquaculture ponds. These studies will include determining fertilization schedules for these ponds, as well as polyculture systems to promote good water quality. Rainfed ponds differ substantially from our more typical experimental ponds in that they receive water only during the rainy season. Evaporation concentrates nutrients and may deteriorate water quality during the dry season. In addition to the emphasis on northeast Thailand, the Philippines work will emphasize two areas of research: field testing of selected tilapia strains, and outreach of CRSP results in the Philippines.

Some of our work will continue to refine the understanding of tilapia culture ponds. These experiments will be conducted at AIT or Bang Sai. We have four such experiments planned: three from University of Hawaii (studies 5, 6, and 8) and one from University of Michigan (study 3). The Hawaii work will continue to focus on nutrient balance in ponds, examining carbon balance and carbon exchange in our ponds. In addition, there will be additional work evaluating polyculture in semi-intensive ponds, using carp as the secondary species and examining the effects of carp foraging on water quality in ponds with acid sulfate soils. The University of Michigan will further evaluate finishing systems for tilapia, with the goal of producing larger tilapia for market by combining supplemental feeding with open pond fertilization. This is the next step in the continuing examination of supplemental feeding strategies for tilapia culture.

While the above experiments have been described as Hawaii or Michigan experiments, they are actually proposed in collaboration. Our collaboration will be enhanced by the presence of both a Michigan and Hawaii Principal Investigator on site during this work plan. The university proposing the work and responsible for the completion of it is the one listed in this description, but both universities share ideas and data, as well as personnel, for the completion of CRSP studies.

Thailand Study 1: Fertilization Schemes in Rainfed Ponds

Objective: Evaluate the effects of fertilizer application frequency on the dynamics of rainfed ponds.

Significance: Our experiments to date have been on ponds where the water level is maintained by adding water and where there may be some flushing of the water by rainfall. In northeast Thailand and many other regions, rainfall occurs only during the wet season and drought during the dry season. Ponds in this region are dug deeper (to 3 m) to store sufficient water to overcome evaporation until the next rainy season. Regular addition of fertilizer into such a pond can result in overfertilization and mortality of the fish, because evaporation may concentrate these chemicals to undesirable levels.

Hypotheses: That weekly application of fertilizer results in appropriate levels of nutrients in the water to optimize primary production and fish yield. That regular measurement of nitrogen concentration will predict optimal fertilization frequency to maximize primary production and fish yield in the ponds.

Experimental Design: Three fertilization and one depth treatment with four replicates each.

Pond Facilities: These experiments may be done at the Udorn Fisheries Station if new ponds are completed by then, or else at AIT in the deepest ponds available.

Culture Period: Five months during the dry season.

Fish Stocking Rate: Sex-reversed *Oreochromis niloticus* at 2 fish/m^2.

Nutrient Inputs: Treatment 1 will have our "normal" schedule of chicken manure at 250 kg/ha/wk supplemented with urea to bring the total nitrogen to 4 kg/ha/d and nitrogen:phosphorous ratio to 4:1. Treatment 2 will also follow this fertilization schedule but will have water added to maintain depth at 3 m. Treatment 3 will have one fertilization at the initiation of the experiment, then no further additions (the most common current management practice). This treatment will be fertilized at 1000 kg/ha of chicken manure supplemented with urea. Treatment 4 will begin with an initial fertilization of 250 kg/ha of chicken manure with supplements, and will subsequently be refertilized when nitrogen levels become limiting. Weekly assays of nitrogen will be done and fertilization will be done when dissolved inorganic nitrogen for combined water column samples becomes less than 0.5 mg/l. Secchi disk readings will be done at the same time as nutrient assays to determine if Secchi disk data could be used to predict when fertilization is necessary.

Water Management: The ponds will be filled at initiation, then the only addition will be by rainfall.

Sampling Schedule: Standard protocols except as noted. Diurnal sampling every month at four depths. Evaporation rate weekly. Weather data (rainfall, wind speed and direction) daily.

Statistical Methods: ANOVA, regression.

Schedule: Data collection December 1993 to April 1994; technical report August 1994.

Thailand Study 2: Polyculture in Deep Ponds

Objective: Assess the effect of other fish species on the water quality and yield of tilapia and of all fish in deep, rainfed ponds.

Significance: These deep ponds may develop severe stratification and low oxygen in bottom waters, as well as loss of nutrients to the deep hypolimnion. Tilapia alone cannot break up this stratification (although our earlier experiments have shown slightly decreased stratification due to fish in some treatments), and wind is often insufficient to do so. In the absence of rain, the ponds become nearly permanently stratified and develop anoxia. Addition of large bodied fish, like carp, which forage in the sediments and actively swim throughout the water column, may break down this stratification regularly, which will prevent anoxia and improve water quality. Carp may also utilize different food items and improve the yield compared to tilapia alone. The use of fish for mixing is preferred over mechanical devices in many of these low intensity and subsistence ponds.

Null Hypotheses: That the addition of common carp will not affect the stratification or total yield of fish from the ponds, compared to ponds with tilapia alone. That carp density will not affect stratification or total yield.

Experimental Design: Three fish stocking treatments with four replicates per treatment.

Pond Facilities: 12 earthen ponds at Udorn (if the newly constructed ponds are completed by then) or at AIT in the deepest ponds available.

Culture Period: Five months in the dry season.

Fish Stocking Rate: Sex-reversed *Oreochromis niloticus* at 2 fish/m^2. Three treatments will differ in stocking density of common carp, with 0 carp in treatment one, 500 carp per hectare in treatment 2, and 1000 carp per hectare in treatment 3. Carp will be stocked at 500 g in size.

Nutrient Inputs: All treatments will receive fertilizer application at the optimum frequency and rate determined in Study 1.

Water Management: Initial filling of the ponds, then addition only by rainfall.

Sampling Schedule: Standard protocols except as noted. Diurnal sampling every month at four depths (stratification can be quantified by top to bottom differentials in O_2 or temperature, as well as depth of the thermocline if one develops). Include Secchi disk depth in diurnals. Evaporation weekly. Weather data daily.

Statistical Methods: ANOVA.

Schedule: Data collection December 1994 to April 1995; technical report August 1995.

Thailand Study 3: Finishing System for Large Tilapia

Objective: Determine techniques for producing tilapia at 500 g market size with the best efficiency.

Significance: Tilapia fetch a much higher price per kilogram when sold at 500 g compared to 250-300 g common in our pond grow outs. However, growth declines at such a large size in semi-intensive ponds, and growing these fish to large market size may take much too long and be too costly. The use of supplemental feeds can accelerate this growth, and caging of tilapia in ponds for the final growout can efficiently produce a number of large tilapia while still utilizing the nutrients produced from the feeding to grow smaller tilapia in a semi-intensive system. Such a system, similar to our earlier work on catfish-tilapia polyculture, would allow small scale farmers to use one pond to efficiently grow young tilapia and still feed older tilapia for a larger final size. Cost/benefit analyses will also allow a comparison of this caging technique to earlier at-large techniques of supplemental feeding.

Null Hypotheses: That stocking density in a cage will not affect growth or yield per fish in the cage. That cage density or stocking density will not affect growth or yield of fish in the pond. That stocking density and cage density will not affect water quality in the pond.

Experimental Design: Four caging treatments with a similar stocking density of young tilapia at large in the ponds and three replicates per treatment.

Pond Facilities: 12 earthen ponds (400 m^2) at AIT or Bang Sai.

Culture Period: Three months.

Fish Stocking Rate: Sex-reversed *Oreochromis niloticus* will be stocked at large in the ponds at 2 fish/m^2. The four treatments will include two densities of fish stocked per cage (25 or 50 fish per m^3) and two densities of cages (4 or 8 cages per pond). Cages will be 1 m^2 in size. Tilapia stocked in cages will average 250 g in size.

Nutrient Inputs and Feeding Rate: All tilapia cages will be fed at 3% body weight per day. Fertilizer rates will be adjusted to appropriate N:P ratios using the bioassay system biweekly. Total nutrient application rate will be dependent on the major nutrient input from supplemental feeding, but will be similar to our optimal rate of fertilization (4 kg/ha/d of N).

Water Management: Replace evaporation and seepage losses weekly.

Sampling Schedule: Standard protocols except as noted. Intensive measurement of physical, chemical, and biological parameters every two weeks. Nutrient bioassay every two weeks.

Statistical Methods: ANOVA.

Schedule: Data collection June 1993 - September 1993; technical report August 1994.

Thailand Study 4: Outreach Assistance

Objectives: Link our research experiments with the outreach program at AIT, which will provide better interaction between our experiments and farmer needs.

Significance: We have continued to refine our fertilization protocols and stocking systems without major input from farmers. This sort of work could continue indefinitely, but becomes more site specific as we proceed from the general concept to the refinements we make. The outreach program at AIT is now working in northeast Thailand to understand farm systems for fish there and to provide assistance in the extension of aquaculture information to these farmers. The outreach program lacks ability to conduct experiments on which to base advice. The CRSP hopes to interact directly with AIT outreach to begin adaptive management systems to speed the delivery of advice to farmers. We propose to post a research associate (in-country hire) and an assistant at Udorn to begin this process. These people will work with AIT staff to identify research needs, and the CRSP staff will then conduct short term experiments to obtain preliminary information on these needs. The preliminary information will be used by outreach staff as well as by us in planning of our future controlled experiments within the CRSP. This type of research and management interaction is similar to Holling's idea of adaptive management, and will speed up the delivery of information to extensionists and farmers as well as improve the usefulness of our major experiments.

Experimental Design: This proposal is not for a typical pond experiment but rather for a new means of conducting experiments and providing assistance. We propose to leave time available for the research team to use adaptive management to plan and conduct major and minor experiments. These experiments will be difficult to propose through our current means, but will be much more meaningful in the provision of assistance to outreach programs and extension. We propose to initiate this effort in this work plan and to provide reporting on its success and modify it as needed for the future.

Initial Research Ideas: Several initial themes have already emerged, including work on the removal of turbidity from pond water, stocking strategies to reduce predatory impacts from fish in non-drainable ponds, evaluation of the frequency and intensity of fertilizer input in rainfed ponds, and evaluation of polyculture as a manager of water quality in rainfed ponds. Two of these experiments are the subject of our major studies in this work plan, while the other two may be the focus of local, short term adaptive experiments. Other short-term experiments will likely develop from the needs of outreach, which is now contacting and interviewing farmers and providing advice.

Pond Facilities: 16 deep ponds to be constructed at Udorn Fisheries Station, as well as many ponds currently available there.

Thailand Study 5: Carp/Tilapia Polyculture on Acid-Sulfate Soils

Objectives: Determine the effects of adding common carp in a tilapia monoculture on:

- Fish production - tilapia production, carp production and total production
- Nutrient dynamics including concentration of nitrogen, phosphorus and carbon (alkalinity)
- Turbidity
- Primary productivity.

Significance: CRSP research in Thailand has concentrated on the dynamics of *Oreochromis niloticus* monocultures. As *Oreochromis niloticus* is primarily a planktivore, the addition of the benthic detritivore *Cyprinus carpio* should lead to increased system productivity through the conversion of currently unutilized benthic matter into fish flesh. The inclusion of carp in the culture system may also increase the recycling rate of nutrients by resuspension of settled organic matter as a result of the stirring activities of the carp (Hopkins and Cruz, 1982). This stirring activity may also affect stratification, particularly in deep ponds. Thailand Study 2 will examine carp/tilapia polycultures in deep ponds in northeast Thailand.

Although adding carp to a tilapia monoculture has the potential to increase yields, it may also have the potential to seriously decrease yields if the pond has acid sulfate soils. Carps could suspend acidic soil into the water column thereby leading to decreases in alkalinity and subsequent carbon limitation. Also, suspension of muds high in aluminum and iron could, under acidic conditions, lead to removal of phosphorus from the water column. Both carbon and phosphorus limitation could decrease yields. Therefore, a separate study to examine the effects of polyculture on water quality and yield in ponds with acid soils is necessary.

Null Hypotheses:

- Common carp will have no effects on the production of tilapia.
- Common carp will have no effect on visibility or primary productivity.
- Common carp will have no effect on pH or alkalinity.

Experimental Design: Five treatments with three replicates in two blocks. Two replicates in block 1 and one replicate in block 2. The treatments are:

- Tilapia only at 2 fish/m^2
- Tilapia at 2 fish/m^2 plus common carp at 0.1 fish/m^2
- Tilapia at 2 fish/m^2 plus common carp at 0.3 fish/m^2
- Tilapia at 2 fish/m^2 plus common carp at 0.5 fish/m^2
- Tilapia at 2 fish/m^2 plus common carp at 0.7 fish/m^2.

Pond Facilities: Fifteen ponds at AIT in two blocks (ponds 1 - 10 are block 1 and ponds 11-15 are block 2).

Culture Period: Five months.

Fish Stocking Rate: Sex-determined fingerling *O. niloticus* at 2 fish/m^2 and *Cyprinus carpio* fingerlings at rates specified in the experimental design.

Nutrient Inputs: Chicken manure at 250 kg dry matter/ha/wk supplemented with urea and TSP to attain rates of 4 kg N/ha/d and 0.8 kg P/ha/d.

Sampling and Water Management: Standard protocols except for detailed water sampling/analyses which will be conducted every two weeks.

Statistical Methods: ANOVA and regression as appropriate.

Schedule: Data collection, October 1993 - March 1994; technical report, September 1994.

Thailand Study 6: Management of Carbon Dioxide Balance for Stability of Total Alkalinity and Phytoplankton Stocks in Fertilized Fish Ponds

Objectives:

1. Further examine the association among the nature of fertilizer inputs, net CO_2 balance, and total alkalinity concentrations in pond water during typical PD/A CRSP pond fertilization experiments.

2. Examine the potential for management of decreasing TA by interim addition of soluble carbonate.

3. Document relationships among medium term net CO_2 balance, trends in TA, and fish growth and production.

Significance: Stability of phytoplankton stocks and photosynthetic activity is important to successful pond culture in general and to fertilizer-based strategies in particular. Large phytoplankton stocks in fertilized ponds are often unstable. Low total alkalinity (TA) can limit photosynthesis in ponds and thus contribute to instability.

Because of the importance of pond soil conditions in TA management, the best practical approach is to analyze and condition the soil before ponds are filled, as extensively discussed by Boyd (1990). However, TA can change substantially during growth cycles in ponds which have received appropriate soil conditioning. CRSP observations in Thailand have documented that ponds fertilized at high rates with purely chemical fertilizers often exhibit decreasing TA during five month growth cycles, sometimes to levels well below 20 mg/L $CaCO_2$. In recent trials in Honduras, TA decreased in chemically-fertilized ponds as seen in Thailand, while increases were observed in manured ponds. Green et al. (1989; Honduras Cycle II) found final TA only slightly higher than initial levels in chemically-fertilized ponds, while heavily manured ponds showed marked increases (>400%). Manured ponds also exhibited increasing TA during Cycle III in Honduras.

Presuming that pond soils have been properly prepared, the most likely explanation for these changes in TA relates to the interaction of dissolved calcium ion (Ca^{+2}) and soil $CaCO_3$ with medium-term (weeks to months) net production or uptake of forms of CO_2. The common presumption is that addition of CO_2 to water by respiration, and photosynthetic removal of CO_2 forms, do not in themselves affect TA. This is true if only the aqueous CO_2 system is considered (Smith and Key 1975). However, consideration of hardness and $CaCO_3$ reveals potential effects of medium term CO_2 balance on TA. As Boyd (1990, p. 43) says, "In many waters, calcium ion is associated with bicarbonate and carbonate ions, so when carbonate increases to an appreciable concentration, calcium carbonate will precipitate because this compound is relatively insoluble." Wetzel (1975) discusses photosynthetic effects on TA and $CaCO_3$, pointing out that effects can rarely be seen over a 24 hour period except under extreme conditions (which do not occur in ponds). Over longer periods, however, changes in the concentration of carbonate ion (CO_3^{-2}) through the CO_2 system equilibrium, associated with consistent net production or removal of CO_2, are the likely mechanisms for the observed changes in TA during growth cycles.

The effect of photosynthesis and respiration on TA is accomplished through net pH changes through extended periods, which in turn determine the abundance of carbonate and the solubility of $CaCO_3$ (Harvey 1966, Wetzel 1975). Net CO_2 accumulation lowers pH, converting carbonate ion to bicarbonate ion and making $CaCO_3$ more soluble, thus increasing TA by dissolving $CaCO_3$. Similarly, net photosynthetic removal of CO_2 species from the water raises pH, which converts bicarbonate to carbonate ion and makes $CaCO_3$ less soluble, thus reducing TA by precipitation of $CaCO_3$. Heterotrophic ponds, those which consistently produce less oxygen than they consume on a daily basis and produce more CO_2 than is fixed by autotrophs (negative NPP), should exhibit increasing TA with time (as observed), provided that a sufficient fraction of the excess CO_2 remains in the pond (some will diffuse out at night), and that sufficient $CaCO_3$ is present to support continuous dissolution. Autotrophic ponds (consistently positive NPP) should exhibit decreasing TA with time, provided there is sufficient dissolved calcium to support continued precipitation.

Therefore, methods for interim management of TA are of both theoretical and practical interest. This study addresses the question, "How can decreasing TA best be managed in fertile ponds during a growth cycle?" This experiment will document the temporal trends of TA in 12 ponds, and quantify the effect of interim addition of soluble carbonate on downward trends in alkalinity. This experiment will begin the larger-scale approach of the Thailand project to enhanced understanding of carbon cycles in ponds and illustrate the level of complexity in alkalinity regimes to be expected at this site. Although soil analyses will be performed before and after the experiment, the primary focus is on the water column during this initial trial.

Hypotheses: Nature of fertilizer material has no effect on amount and temporal pattern of total alkalinity under these conditions.

Total alkalinity is unrelated to phytoplankton stock and net CO_2 balance when concentrations are greater than 50 mg/L as $CaCO_3$.

Fish growth, survival, and yield are unrelated to nature of fertilizer material and net CO_2 balance when TA is greater than 50 mg/L as $CaCO_3$.

Experimental Design: Twelve 0.04 ha ponds at the Asian Institute of Technology will be stocked with fingerling male *O. niloticus* at a rate of 20,000 fish/ha, maintained at 1.0 m depth weekly, and operated for 150 days. Six ponds will be fertilized with urea and phosphates at 28 kg N/ha/week with N/P = 5.0; six others will be fertilized with an isonitrogenous (and of similar N/P ratio) combination of 200 kg/ha/week chicken manure and chemical fertilizers. Within each of these two treatments, three ponds will receive no inputs of carbonate (unless some is contained in manure, which will be analyzed) during the trial, while three will receive additions of sodium carbonate (Na_2CO_3) every two weeks in proportion to observed TA decreases, when these occur. [If the agreed-upon global experiment can be performed by modest adjustment of the stocking rates and absolute amounts and ratios of N and P above, such adjustments will be made for all ponds, and the ponds receiving no carbonate additions will serve as the Thailand project's performance of that experiment.]

Pond Facilities: 12 - 0.04 ha earthen ponds

Culture Period: 150 days

Stocking Rate(s): 20,000 fish/ha

Water Management: Depth to 1.0 m weekly

Other Inputs: Inorganic-only treatment: 4 kg N/ha/wk @ N/P = 5.0; urea and TSP. Organic combination treatment: chicken manure at 200 kg/ha/wk, urea to make up N = 4 kg/ha/wk, TSP to approximate N/P = 5.0.

Sampling Plan: Standard protocols except: Analysis of chlorophyll *a* concentration and total alkalinity will be performed weekly on two depth-integrated samples from each pond; samples will be taken two days before scheduled fertilization, with results and carbonate additions to be available at fertilization. Net production and respiration will be assessed weekly by diel sampling (0600, 0900, 1200, 1500, 1800, 2200, 0600) of DO and pH at three depths (5, 35, 80 cm). Soil pH and base-saturation will be analyzed before filling and after draining of ponds at three locations from each pond. Other analyses will be performed according to recent "standard" protocols, or as necessary for the global experiment.

Statistical Methods: ANOVA, correlation, time series analysis.

Schedule: December 1993 - May 1994.

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Thailand Study 7: Diel Cycles of Temperature and Dissolved Oxygen Stratification in Deep Rain-fed Ponds

Objectives:

1. Describe and quantify diel cycles of temperature and DO stratification in the deep rain-fed ponds of current focus in the AIT Outreach activities.

2. Compare these patterns with those of shallower ponds typical of CRSP experiments.

3. Integrate this information with pond-management requirements under investigation by other program components.

Significance: In regions of large seasonal differences in rainfall, particularly where no water inputs can be made to ponds during the dry season, such as the Udorn Thani region of northeast Thailand, culture ponds are constructed sufficiently deep to hold amounts of water which will sustain evaporative losses through growth cycles. The Asian Institute of Technology's Outreach Project is collaborating with the Thailand CRSP component to extend results of CRSP work to farmers in this area. The University of Michigan component is constructing a set of experimental ponds on the DOF station in the area.

The rain-fed ponds' greater depth (2-3 m) suggests that density stratification will be more severe than is common in shallower ponds, and therefore less often dissolved by convective overturn at night or by wind-induced mixing. This makes oxygen depletion in the hypolimnion more likely. Management strategies involving artificial mixing are under consideration by the principals of the Outreach Project. This study aims to provide quantitative information on the characteristics of diel temperature stratification to be expected, which will later be used to plan mixing strategies. Similar observations can then be used to evaluate the effects of mixing methods employed.

The study of Szyper and Lin (1990) and subsequent observations at AIT have characterized the stratification patterns to be expected in ponds of 0.6 to 1.6 m depth. For example, on relatively still, sunny days during the dry season, surface warming initiates stratification within an hour after sunrise. Temperature differences between top and bottom increase through 1500 to 1600 hours, reaching 7 to 8° C. Within 10 to 20 cm of the bottom, temperatures typically change little during day or night. This illustrates the isolation of bottom waters, which in some cases exhibited dissolved oxygen concentrations (DO) less than 1 mg/L throughout the diel cycle, with the exception of increases amounting to a few mg/L between midnight and 0600 hours due to convective mixing with the surface waters. Detailed temperature profiles showed that under these conditions, the hypolimnion, the depth layer isolated below the thermocline, constituted more than half the pond depth.

These observations dictate the need for quantitative information on ponds to be mixed artificially, because mixing, unless timed and located properly, could reduce whole-pond DO levels to markedly below optimal, and possibly below tolerance limits, for cultured animals. In addition, mixing can have profound effects on primary production, which is the primary source of nourishment to the animals in fertilizer-based production strategies.

Experimental Design: Ponds from the experimental set will be monitored during trials designed by the University of Michigan component of the project. Fours periods of 2 to 7 days during a year, selected to include two seasonal extremes and the intermediate periods, will be studied. During each study period, a primary pond will be monitored for temperature and DO cycles with an automated monitoring system similar to that used at AIT. Temperature is monitored by thermocouples deployed on a plastic pipe suspended from a float; DO is recorded in water pumped from the pond through a receiving chamber of plastic pipe on the pond bank. The system will include monitoring of incident solar irradiance and wind speed at 0.5 m above water level. At least one neighboring pond will be monitored for temperature profiles simultaneously, in order to document variation among ponds, which has been small for these properties at AIT. Diel temperature and density patterns at 6 - 10 depths will be recorded every 30 minutes, as will DO at three depths in the primary pond. The data set will be analyzed in addition for net production and nighttime respiration by the diel curve method.

All other aspects of pond preparation, stocking, and management will be determined by the experimental objectives of the UM component.

Hypotheses:

1. Stratification will be more severe (greater temperature difference between top and bottom waters) and more persistent than that recorded in ponds of 0.6 - 1.6 m by these methods previously, with more extensive hypolimnion development.

2. Under high incident irradiance, more severe stratification (top bottom temperature difference at noon) will be associated with reduced daytime and diel net oxygen production.

Pond Facilities: one rain-fed earthen pond.

Culture Period: P_{exp} (determined by primary experiment).

Stocking Rate(s): P_{exp}

Water Management: P_{exp}

Other Inputs: P_{exp}

Statistical Methods: t-tests, ANOVA, correlation and regression.

Schedule: December 1994 - April 1995, or during other appropriate experiments.

References:

Szyper, J.P., and C.K. Lin, 1990. Techniques for assessment of stratification and effects of mechanical mixing in tropical fish ponds. Aquacultural Engineering 9:151-165.

Thailand Study 8: Carbon Dioxide Exchange Between Pond Water and the Atmosphere

Objectives:

1. Quantify the rates of exchange of carbon dioxide between pond water and the atmosphere in fertile fresh water earthen ponds.

2. Compare estimated exchange rates with rates of photosynthetic carbon uptake by pond phytoplankton and with rates of carbon release during community respiration.

Significance: Rates of exchange of dissolved oxygen (DO) and carbon dioxide between pond waters and the atmosphere are often significant components of ponds' budgets for these materials. Because these gases are produced and taken up by pond microbes and cultured animals in respiration and photosynthesis, accurate estimates of these processes must take account of atmospheric exchange. Bottle-incubation methods typically neglected these processes because separate estimates of concentrations in the free pond water would be required and were not made. Free-water assessment of photosynthesis and respiration is based on sequential assessment of pond concentrations through time; exchange is estimated from these data, using in addition wind speeds and temperature-dependent saturation values for the gases (Banks and Herrera, Weisburd and Laws 1990, Boyd and Teichert-Coddington 1992).

Oxygen exchange is routinely estimated in free water studies (Hall and Moll 1975, Green et al. 1989, Szyper et al. 1992), but far less attention has been given to carbon dioxide. If automated methods could progress to the point of short-interval estimates of daytime respiration as well as net concentration changes, for both oxygen and carbon, estimates of gross primary production and total diel community respiration could be made in terms of both elements. It would then be possible to calculate at least the photosynthetic quotient (moles oxygen evolved:moles carbon taken up) for pond phytoplankton communities, which in turn would facilitate study of carbon budgets and other pond processes.

The few reported estimates of carbon dioxide exchange rates have been done in salt- or brackish water ponds, and under conditions of less severe density stratification and lower rates of primary production than is typical of CRSP experiments. There are indications that these rates, while somewhat smaller compared with photosynthesis and respiration than those observed with oxygen, are too large to neglect (Szyper and Ebeling 1993). Good estimates of these rates in typical CRSP ponds will provide baseline data and an opportunity for refinement of estimation methods, both pertinent to the Thailand project's approach to enhanced understanding of carbon cycles.

Hypotheses: Atmospheric carbon dioxide exchange rates are substantial in comparison with rates of primary production and community respiration during CRSP experiments ($\geq$ 10% of community rates more than 50% of the time).

Experimental Design: An 0.04 ha earthen pond used as part of a CRSP experiment at AIT (first priority: Study 6) will be monitored at three depths (5, 35, 80 cm) every 30 minutes for DO, pH, and temperature on three dates, once every two weeks during a growth cycle, bracketing the day of diel manual sampling. The system records temperature and pH in water samples pumped from the pond to a receiver of plastic pipe on the bank, which contains a thermocouple and pH electrode. Total carbon dioxide, which includes aqueous carbon dioxide, carbonic acid, bicarbonate ion, and carbonate ion, is calculated from pH, temperature, and total alkalinity (the latter analyzed manually). Primary production and community respiration are calculated by the free water method from changes in total carbon dioxide. Atmospheric exchange is calculated from wind speed, the aqueous carbon dioxide concentration (derived from the system equilibrium), and temperature-dependent saturation values (Weisburd and Laws 1990). Results of both automated and manual diel sampling programs will be compared, with attention to the relative importance of atmospheric exchange at various stages of bloom development.

Inputs, management, and sampling for other quantities will be determined by the protocols of the primary experiment.

Pond Facilities: one 0.04 ha earthen pond.

Culture Period: $P_{exp.}$ (determined by primary experiment).

Stocking Rate(s): $P_{exp.}$

Water Management: Depth to 1.0 m weekly.

Other Inputs: $P_{exp.}$

Sampling Plan: Standard protocols except: Diel sampling (0600, 0900, 1200, 1500, 1800, 2200, 0600) of DO and pH at three depths (5, 35, 80 cm) every two weeks.

Hypothesis: Atmospheric carbon dioxide exchange rates constitute much greater percentages of community rates when the community rates are low at the beginning of experiments.

Statistical Methods: t-tests, ANOVA, correlation and regression.

Schedule: December 1993 - May 1994, or during other appropriate experiments.

References:

Banks, R.B., and F.F. Herrera. 1977. Effect of wind and rain on surface reaeration. J. Environ. Eng. Div., ASCE 103:489-504.

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Thailand Study 9: Yield Trials with Genetically Selected Tilapia

Objective: Determine the growth and yield of genetically-improved tilapia in ponds fertilized according to the most current CRSP guidelines.

Significance: During early 1993, CRSP research in the Philippines will include preliminary yield trials of genetically selected *Oreochromis niloticus* produced by Genetic Improvement of Farmed Tilapias (GIFT) project[1] and the FAC/University of Wales Swansea Research Project on Genetic Improvement of Tilapia. The GIFT fish are cross-bred strains of *O. niloticus* while the FAC/Swansea fish have been subject to hormonal and genetic manipulation to produce YY "supermales." As these early trials will be preliminary in nature, it will be essential to repeat them during the first half of the Seventh Work Plan.

Null Hypothesis: There will be no differences in growth rates and yields.

Experimental Design: Five treatments with three replicates. The treatments are:

- Philippine strain *O. niloticus*
- Thailand strain *O. niloticus*
- GIFT *O. niloticus*
- YY-male *O. niloticus*
- Communal culture with tagged fish from the four groups.

Pond Facilities: Fifteen 500 m^2 ponds at FAC.

Culture Period: Five months.

Fish Stocking Rate: Sex-determined fingerling *O. niloticus* at 2 fish/m^2.

Nutrient Inputs: Most current CRSP fertilization guidelines.

Sampling and Water Management: Standard on-farm protocols.

Statistical Methods: ANOVA and regression.

Schedule: Data collection, October 1993 - March 1994; technical report, September 1994.

[1] The Genetic Improvement of Farmed Tilapias (GIFT) project is a cooperative effort led by ICLARM. Other major participants include the National Freshwater Fisheries Technology Research Center of the Philippine Bureau of Fisheries and Aquatic Resources and the Freshwater Aquaculture Center of Central Luzon University.

Thailand Study 10: Philippines Outreach

Objective: Link our research experiments with outreach and extension programs in the Philippines. This should lead to refinement of CRSP recommendations to reflect local conditions in the Philippines and the adoption of CRSP fertilization guidelines in the Philippines.

Significance: The CRSP in Thailand started an effort to include major input from fish farmers in our research in 1992. Given the short time period, these efforts have been relatively successful. CRSP activities in the Philippines started in 1991 to conduct on-station trials of CRSP guidelines. By 1993, on-station trials will need to be complemented by on-farm trials. In this way, we hope to adapt the CRSP guidelines to the conditions facing Philippines farmers.

Experimental Design: The experimental activities will consist of three phases. First, a study will be conducted using already available information to determine if the CRSP fertilization guidelines are socially acceptable and economically viable under Philippine conditions. Second, a mechanism to improve and disseminate CRSP guidelines will be developed by the CRSP in cooperation with fish farmers, potential fish farmers and extension personnel. Lastly, field tests of the CRSP guidelines will be started at selected private farms.

Pond Facilities: At least 10 private fish ponds.

Culture Period: To be determined based on local fish size preferences and estimated returns.

Fish Stocking Rate: To be determined based on local fish size preferences and estimated returns.

Nutrient Inputs: Based on most current CRSP fertilization guidelines.

Sampling and Water Management: Standard on-farm protocols.

Schedule: Research activities, June 1993 to July 1994; technical report, September 1994.

Data Base Management

Cooperating Institutions:

University of Hawaii
Dr. Kevin Hopkins
Assistant Data Base Manager, to be named
All CRSP institutions
Other institutions as identified

Objectives:

- Maintain the current relational data base.
- Perform preliminary data analyses.
- Review data from field stations for conformance to formats and identify outliers (>3 s.d. from the mean) values. Transmit this information to principal investigators for verification and/or correction.
- Enter the data into the CRSP relational data base as data are received; update data base as corrections are received.
- Make data available to the DAST and other interested persons in several different formats upon request.
- Print data tables for data reports upon request.

Significance: The CRSP has developed a data base management system to store and retrieve most data collected during CRSP experiments since the start of the CRSP. The data base was considered to be a Program Management Office function until March 1993 when it was formally decided to transfer the data base to the University of Hawaii at Hilo. The data base is a resource of major importance for researchers and is available upon request. By looking at large amounts of data simultaneously, it has been possible to detect trends which are not readily apparent with smaller data sets.

Schedule: The data base was transferred to the University of Hawaii at Hilo in April, 1993. In May 1993, the data base was installed on UHH computers and was operational. Data will be entered as it is received. Reports will be issued upon request.

Data Analysis and Synthesis Team

Cooperating Institutions:

Oregon State University
Dr. James E. Lannan
Dr. John P. Bolte
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Mr. Shree S. Nath
Mr. Douglas H. Ernst

University of California at Davis
Dr. Raul H. Piedrahita
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Introduction:

Research work by the Data Analysis and Synthesis Team (DAST) concentrates on two primary areas namely mechanistic and empirical modeling of pond dynamics, and development of pond management guidelines. Mechanistic models typically allow for a better understanding of the system being modeled because they attempt to describe the underlying mechanisms of different physical, chemical and biological processes that occur in the system, but their predictive power may be limited by exhaustive data requirements and an incomplete description of the system. Empirical models on the other hand may exhibit better predictive capabilities than mechanistic models because they are determined by the best fit to real data, but often lack heuristic power. In practice, however, generalizations about mechanistic and empirical models are not always true, in that both model types exhibit varying degrees of predictive and heuristic power. Previous research by the DAST suggests that a combination of empirical and mechanistic models can be used to direct future research and form the basis for recommending pond management strategies. Therefore, studies proposed by the DAST for the Seventh Work Plan will continue to emphasize development, refinement and use of different types of models to improve understanding of pond ecosystems, to conduct realistic long-term simulations of water quality and fish yields, and to generate recommendations for pond management. An integral part of DAST research will continue to be the use and design of computerized tools, both for model construction and decision support.

The models that have been developed to date by the UCD component of the DAST adequately simulate various aspects of water quality (e.g., net primary productivity, temperature and dissolved oxygen stratification) from modest data inputs. However, community respiration dynamics is poorly understood (mainly due to the lack of suitable instruments to measure this parameter in the field), and this factor has hindered accurate determination of gross primary productivity in ponds. Equipment that has already been developed by the DAST in conjunction with other CRSP researchers will be refined for field use, and the resulting data used to fine-tune existing primary productivity models.

Current DAST models are deterministic in nature, implying that model inputs are known with complete certainty and that the same results are obtained for a given set of input variables. Thus, variability in the input variables is not accounted for in these models.

Climate data (solar radiation, cloud cover and wind speed), which are stochastic in nature, form a critical input into existing models of pond dynamics. Although historical data from the CRSP database or from meteorological stations in various countries can be used in these models for long-term forecasting of pond behavior and consequently fish yields, this approach is of very limited use because of the substantial variability in climatic characteristics. Combining deterministic models that have already been developed with stochastic models that use random values drawn from probability distributions of climatic characteristics, would be a much more powerful tool to simulate pond performance over the long-term (e.g., one or more growing seasons). Output from such models will allow the generation of probability distributions of water quality and fish yields, and would be valuable in quantifying the risk or probability of obtaining certain fish yields from a given set of management practices. This information can then be incorporated into decision making processes.

The pond management guidelines that have been developed by the OSU component of the DAST are currently restricted to fertilizer application, and have been implemented in the form of a computerized decision support system (DSS) called PONDCLASS. Preliminary field testing of these fertilization guidelines by CRSP participants has given satisfactory results. A more thorough testing of the guidelines at all the major CRSP sites is proposed for the Seventh Work Plan, results of which should generate important feedback for refinement of the models that are presently used. The guidelines currently require estimates of primary productivity at a given site to be available before recommendations can be generated. Such data may not always be available, and may restrict long-term planning for an aquaculture facility. Therefore, available DAST models that can be used to predict net primary productivity from light intensity, pond turbidity and depth will be modified for use in long-term simulations.

Information on other methods such as supplemental feeding, stocking/cropping strategies and water quality maintenance that can be used to enhance fish yields from aquaculture ponds has not been synthesized into comprehensive pond management guidelines. The most important variables that affect fish growth are food availability, stocking density, fish weight, temperature, dissolved oxygen and unionized ammonia, all of which can be controlled (at least to some extent) in pond aquaculture by different management practices. Therefore, it should be possible to simulate the effects of different management practices on fish growth by the use of a bioenergetics model that includes the above variables. The output from such a model can indicate the degree of resource utilization efficiency possible for various management scenarios (e.g., fertilization only, fertilization+supplementary feeding).

Pond management practices attempt to modify physical and chemical conditions of the pond such that the target fish species are able to realize an optimal growth rate. The term optimal is intended to imply that the realized growth rate is not necessarily the maximum growth rate possible for the species in question, but one that is the most economical. Thus, it is also essential to analyze the costs and benefits that accompany any recommended practice(s). Because of geographical variability in the natural productivity of ponds, and due to differences in resource costs and availability among potential sites for pond aquaculture, management practices that are economically optimal for one site may not be so at another location. Therefore, it is important to develop a generalized (site-independent) framework that allows for a bioeconomic analysis of pond management practices, and that can be adapted for use wherever pond aquaculture is practiced.

A Global Experiment that will be conducted at all the CRSP sites will also form part of the DAST activities for the Seventh Work Plan. The experiment will allow comparisons to be made among sites, improve understanding of how site characteristics affect pond performance and fish yields, and provide an opportunity to refine current fertilization guidelines based on thorough field testing.

Six studies are proposed by the DAST for the Seventh Work Plan. The goals of these studies are:

1. To improve understanding of the dynamics of respiration rates in aquaculture ponds, and to develop methods for quantifying these rates.

2. To develop a stochastic model of pond water quality and fish yield.

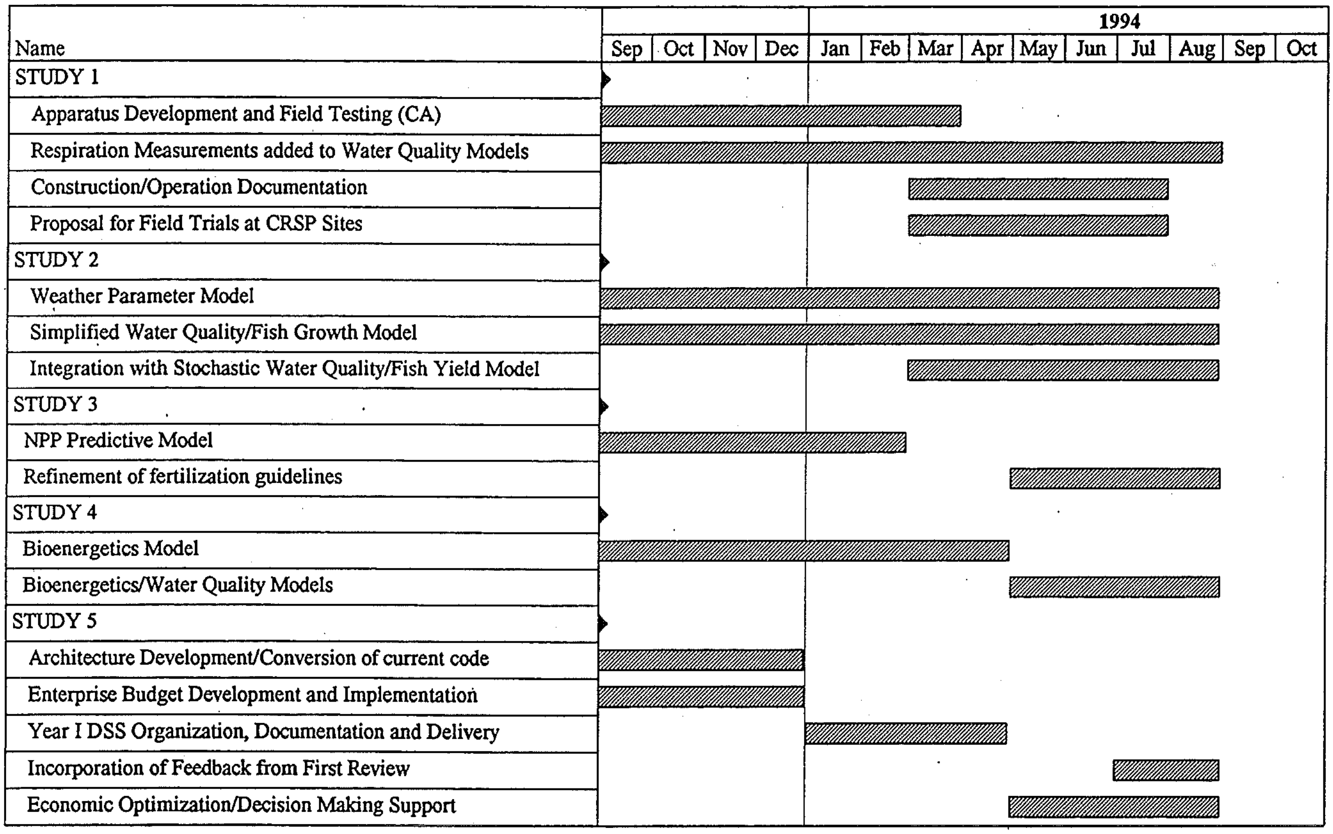
3. To refine current fertilization guidelines, and adapt existing primary productivity models to allow long-term predictions.

4. To develop water quality/fish bioenergetics models for simulation of the effects of management actions on pond production.

5. To integrate water quality/fish bioenergetics models with economic techniques into a comprehensive decision support system for pond management and facility planning.

6. To generate quantitative baseline information on biological and physico-chemical parameters of ponds treated identically at all CRSP sites that will be used for refinement of pond dynamics models, and to field test current fertilization guidelines.

Details of specific activities for Studies 1 to 5 are shown in Figure 1. The schedule for Study 6 is not indicated because the Global Experiment may not be conducted simultaneously at the different sites.



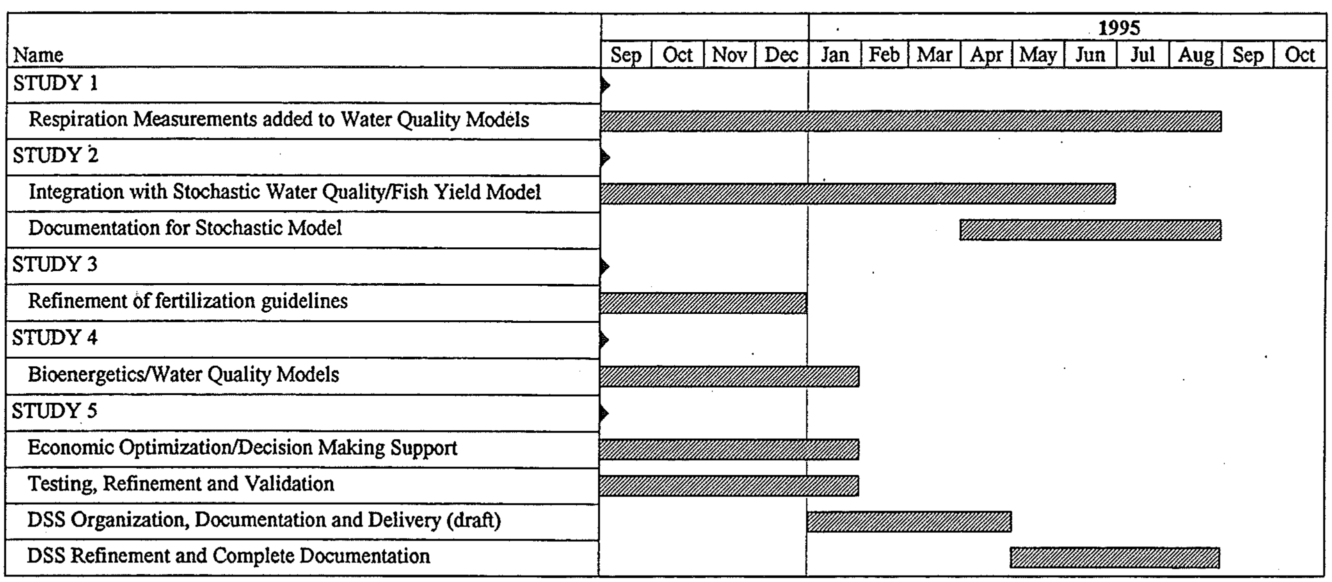


Figure 1. Schecule of DAST activities for 1993-1994 and 1994-1995.

DAST Study 1: Respiration Dynamics in Aquaculture Ponds

Objectives:

1. Refine the existing apparatus for pond respiration measurements.
2. Test and evaluate the pond respiration apparatus developed.
3. Propose field experiments to be carried out at the CRSP sites.
4. Incorporate findings from respiration measurements into existing water quality models.

Significance: Previous DAST studies have resulted in the development of methodologies for the determination of hourly rates of community respiration in aquaculture ponds over the diel period. These methodologies have been applied in field tests at the Mariculture Research and Training Center (MRTC) of the Hawaii Institute of Marine Biology, and have been published (Szyper et al, 1992). These results show clearly the potential of the techniques, but there is a need to continue to refine and develop the equipment and procedures. This study proposes to continue the development of a system for the determination of rates of continuous diel community respiration in aquaculture ponds, for use in analysis of pond dynamics.

Community respiration rates are subject to complex influences by environmental and other factors which are not fully understood. In addition, phytoplankton respiration consists of at least two separate components—endogenous respiration and photorespiration. Endogenous respiration, also termed "dark" respiration is defined by Graham (1980) as "CO_2 evolution or O_2 uptake resulting from the oxidation of substrates ..." This endogenous or "dark" respiration occurs both in the dark and the light. In contrast, photorespiration is defined by Graham (1980) as "the light dependent oxidation of a C_2 metabolite derived from photosynthesis." Because these two processes are separate and distinct, yet occurring simultaneously during light periods, the development of an accurate mechanistic model for the sum of these processes has so far not been achieved. Furthermore, because these respiration processes are influenced differentially by such factors as light, temperature, dissolved oxygen concentration and photosynthesis, and because different phytoplankton species exhibit different respiration responses to these variables, the determination of an accurate respiration function for use in aquaculture ponds containing multispecies is a daunting proposition.

It is apparent from previous work by the CRSP DAST that water column respiration cannot be described accurately by empirical temperature dependent functions. Equally problematic is the common practice of extrapolating nighttime pond respiration rates to daytime periods. What occurs during the daylight hours is subject to speculation, however some laboratory studies have indicated that the light respiration rate appears to be proportional to the primary production rate (Geider and Osborne, 1989). If this is the case, then extrapolating the nighttime respiration rates over the diel period would cause a significant underestimation in total pond respiration, which would then result in significant underestimation of gross primary production.

A system developed by the University of California DAST in conjunction with University of Hawaii CRSP researchers has attempted to estimate community respiration rates in aquaculture ponds continuously over diel periods, by quickly darkening pond water samples of actively photosynthesizing algal cells. By immediately measuring the rate of O_2 consumption during the short time period after darkening, it is possible to get a more accurate estimate of the total community respiration rate during the light period. This technique is consistent with results of mass spectrometric analysis of gas exchange in phytoplankton which have shown an increased respiration rate after exposure to light (Weger et al, 1989). Preliminary results from this initial effort have been published (Szyper et al, 1992). This work follows previous efforts to examine in situ processes by isolating water samples in specially developed chambers. In particular, Dubinsky et al. (1987) developed an apparatus to measure photosynthesis in a well defined light chamber, and Pearson et al. (1984) developed an in situ respirometer for coral. However neither of the previous efforts addressed the specific need for measurement of "light enhanced" dark respiration (Falkowski et al. 1985) in the field. This work also differs substantially from other respiration monitoring systems such as presented by Bott et al. (1978) which do not measure diurnal respiration dynamics.

To date, two systems have been developed and tested. The first system uses conventional dissolved oxygen probes (YSI Model 5739) and stirring devices, and has been field tested at MRTC. Results of the field testing have been good, but the performance is often erratic without constant attention because of the complexity of the system. The second system uses microprobes (Microelectrodes, Inc. Model MI-730) which preclude the need for stirrers and simplifies the mechanical aspects of the system. This second system has yielded excellent results in laboratory experiments, but the probes have proven to be extremely fragile and difficult to handle. For these reasons, we propose the development of a field-ready respiration measurement system based on the YSI probes. The goal is to have a system that can eventually be used at CRSP sites with a minimum of effort. This system would be used to provide site information on respiration and overall primary productivity. Information collected at CRSP sites would be compiled, analyzed, and incorporated in models of dissolved oxygen in aquaculture ponds.

Methods: The pond respiration apparatus developed previously will be refined for measurement of respiration dynamics in a variety of aquaculture ponds. Our intent is to have a system that is self contained and that can be deployed and used simply by connecting it to a data acquisition system and providing some source of DC power. The system needs to be modified to improve sample mixing, simplify probe cleaning and maintenance, and to avoid the effect of pressure surges caused by solenoid valves on dissolved oxygen readings.

The modified system will be tested and evaluated in the lab initially and later in ponds in California and at other sites if possible. Detailed instructions for construction, operation and maintenance of the new system will be developed for distribution to the CRSP sites. A proposal will be presented for an experiment to be conducted at the various CRSP sites using the newly developed equipment. The experiment would consist of an intensive, short-term data collection regime that would include diel measurements of community respiration. The experiments would be carried out in CRSP ponds without modification of ongoing pond management and data collection protocols.

Schedule: The equipment will be field tested in California by March, 1994. Detailed instructions for construction and operation of the equipment will be distributed to the CRSP sites by July, 1994. A proposal will be presented at that time for a CRSP experiment to be conducted at all sites using the equipment developed. Model development and quantification of respiration rates will be ongoing as the equipment is tested and the data are collected.

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DAST Study 2: A Water Quality/Fish Yield Model with Stochastic Inputs

Objectives:

1. Develop simple probability distributions for weather parameters at PD/A CRSP sites.
2. Develop simplified relationships between those distributions obtained under objective 1 and water quality parameters.
3. Incorporate the relationships developed under objectives 1 and 2 into a probabilistic model of pond water quality and fish yield.

Significance: Models of aquaculture ponds have been developed following the traditional methods and techniques used for simulation of other ecosystems (Piedrahita 1992). The processes involved are usually considered to be mechanistic in nature without any consideration of non-deterministic behavior. Mechanistic models are based upon a hypothesis about the nature of a system (Riggs 1963; Spain 1982) and "the deterministic parameter behavior" means that the model uses fixed values for model as inputs and produces the same results from a given set of input variables (Meyer 1980; Piedrahita 1984; Svirezhev et al. 1984). In such models, the state of a given variable at any time is determined entirely by previous states of that variable and the other variables upon which it depends. Models which consider parameters with probabilistic behavior (like solar radiation, cloud cover, and wind speed) are necessary to attempt a better description of the processes occurring in aquaculture ponds. Improved knowledge of pond processes would permit more efficient use of available resources. At the same time, management of the ponds would be established on a more rational basis, and the possibility of catastrophic events due to mismanagement would be greatly reduced.

A stochastic (probabilistic) model uses values selected randomly from probability distributions of model parameters. Repeated calculations with the same input variables will, therefore, yield different output variables with every calculation. Probabilistic models are generally more complex than deterministic models because of the mathematics involved in formulating the random process (Cuenco 1989). Some idea about the mathematics involved in stochastic water quality modeling can be seen in Straskraba and Gnauk (1986) and Finney et al. (1979).

Griffin et al. (1981), incorporated both wind and cloud cover as variables randomly determined using a uniform distribution into a bioeconomic model, and Sadeh et al. (1986), made temperature variation a stochastic factor in scheduling of stocking and harvesting in a grow-out pond in Texas in their model, but there are few other aquaculture models allowing stochastic behavior of their parameters, despite other works treating weather parameters as stochastic phenomena (Goh and Tan 1977; Gordon and Hochman 1984; Salcedo and Recio 1984; Amato et al. 1986; Racsko et al. 1991).

The project proposed will build on the models of water quality and fish production in PD/A CRSP aquaculture ponds incorporating solar radiation as a random variable into the mathematical model framework. This work will provide important information for the continued development of models in water quality and fish production.

Current models of water quality and fish production in aquaculture ponds are based on deterministic relationships between atmospheric, chemical and biological parameters within the ponds. The relationships and model proposed under this project would constitute a first attempt at the development of a probabilistic model that can be used to project water quality and fish production over a growing season. The model would be valuable in that risk or probability factors would be associated with particular fish yields under a given set of management practices. This information would then be available for decision making purposes.

Methods: The PD/A CRSP data on solar radiation will be analyzed to develop probabilistic functions based on historical values of solar radiation at PD/A CRSP sites. These functions will then be incorporated into the mathematical model framework of water quality and fish production models.

The solar radiation function will be computed from the solar radiation PD/A CRSP data as a function of time and location by using time series corrected by a probability function that indicates the likely extent of cloud cover for a particular date. This function will then be incorporated into the model as a randomly varying parameter. The relationships under objective 2 will be determined using energetic/mass balance approaches incorporating those distributions developed under objective 1.

A simplified model of water quality and fish yield in ponds will be assembled using the expressions and procedures identified under objectives 1 and 2. The model will be executed such that probability distributions of yields are obtained as an output. This will be achieved by running the model many times and sampling from the resulting distributions of the outputs. The model output will then be assembled in a new probability distribution which can be analyzed to examine the likelihood of a particular outcome, or the likelihood of exceeding a particular level of production.

Schedule: The methodologies mentioned in objectives 1 and 2 will be developed and tested with one of the CRSP site data sets by August, 1994. Development of the stochastic model will be an on-going project. The first version of the model will be completed and tested by August, 1995.

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DAST Study 3: Refinement of CRSP Pond Fertilization Guidelines

Objectives:

1. Modify primary productivity models for use in fertilizer routines of the decision support system.
2. Refine existing fertilizer algorithms based on continued CRSP research.

Significance: Fertilizer calculations in PONDCLASS are based on the expected net primary productivity (NPP) at a site, which is currently a user entry. A recent simulation model developed by the UCD component of the DAST suggests that NPP can be estimated from light intensity, turbidity and depth measurements in ponds. The model in its present form can perform only short-term simulations, but it can be used as the basis for developing a predictive tool that can be used to arrive at expected values of NPP over extended periods (e.g., a full culture cycle or a complete year).

Considerable feedback regarding the assumptions made in the current version of the fertilization guidelines is anticipated from the results of the Global Experiment that will be conducted at all the field sites (see Study 6 below) using the following two treatments: (1) fixed fertilization levels (2) PONDCLASS testing. Assumptions that will be tested in this experiment include the concept of applying fertilizers at fixed levels throughout the culture period (Treatment 1) as opposed to the PONDCLASS rationale of fertilizer addition based on empirical observations on pond productivity, alkalinity enhancement in ponds that are potentially carbon limited and effects of nutrient availability from manures on the quantities and costs of fertilizers added to ponds. Application of lime to neutralize soil acidity in the PONDCLASS treatment will also provide an opportunity to improve liming recommendations. This information together with results from other pond fertilization experiments can be used to further refine the guidelines.

Inclusion of tools that can be used to predict primary productivity in ponds and other refinements of the current fertilization guidelines based on field experiments will allow users to more realistically project fertilizer requirements and costs for an entire culture period. This should enable better planning and economic evaluation of the facility.

Methods: The existing primary productivity model of the DAST will be modified for inclusion in the decision support system as an additional utility for NPP prediction. Anticipated modifications include model recoding to be compatible with the rest of the software, and to allow long-term prediction of NPP. Initially, simplified climate models that have been used to describe photosynthetic processes in terrestial crops will be used to generate light intensity data for the NPP model. It should be possible to include more sophisticated climate models at a later stage based on results of DAST Study 2 to be conducted by the UCD group. Other modifications may include estimation of turbidity from a range of Secchi disk visibilities common in ponds. Typical operating depths for ponds will either be assumed or supplied by the user. The overall NPP model will be continually updated on the basis of ongoing research (see Study 1 above) by the UCD-DAST.

Other changes to the fertilization guidelines will be made on the basis of results from the Global Experiment and other fertilization studies to be conducted by CRSP field personnel. Such changes may include improved capabilities of estimating the quantities of lime required for neutralization of soil acidity and lime enhancement (if needed), and nutrient availability from manures.

Schedule: The initial version of the model for NPP prediction is expected to be completed by February, 1994. Refinement of this model (based on results of DAST Studies 1 and 2 above of the UCD group) together with other changes to the fertilization guidelines will be accomplished from 1 May 1994 to 31 December 1994.

DAST Study 4: Alternate Pond Management Strategies

Objectives:
1. Develop a bioenergetics model for tilapia growth in ponds.
2. Integrate water quality and bioenergetics models into a unified tool for prediction of the effects of supplementary feeding, stocking/ cropping strategies and water quality maintenance on fish growth.

Significance: *Supplementary feeding*: Some or all of the important variables that influence fish growth (food availability, stocking density, fish weight, temperature, dissolved oxygen and unionized ammonia) have been used in previously developed models available in the literature. These models do not specifically address the growth of pond fish like tilapia, or some of the unique features of pond aquaculture. For example, it will be necessary to estimate parameters for temperature, size and spawning effects on tilapia growth. Further, contemporary growth models cannot be directly used to determine feeding rates in fertilized ponds because part of the energetic requirements of the stocked fish is met by natural food. It is generally accepted that supplementary feeding is not required in fertilized ponds until the critical standing crop (CSC) is reached, at which point growth rates begin to decline because natural food becomes limiting (Hepher 1978). Because of the variability in natural productivity of among ponds, CSC's are likely to differ among sites. Therefore, estimation of CSC, given site characteristics, is important in determining when supplementary feeding should commence in fertilized ponds.

The quantity of supplemental feed to be added to ponds to ensure that fish growth is not food limited can be estimated from the relationship between growth and ration size (food consumption), which has been described for some fish species including tilapia (Hepher et al. 1983; Zonnefeld and Fadholi 1991) in the literature. Variables like stocking density, fish weight, temperature, dissolved oxygen (DO) and unionized ammonia (UIA) appear to affect fish growth via their influence on food consumption (Cuenco et al. 1985). Therefore, a model that synthesizes ecological (Hepher 1978) and bioenergetic (Brett 1979; Cuenco et al. 1985) concepts can provide useful information for supplementary feeding in fertilized ponds.

Stocking and cropping strategies: Fish yields can be enhanced by appropriate stocking and cropping strategies which influence the population density in ponds. Effective adoption of this technique requires an understanding of the relationship between fish growth and biomass, because the effect of a standing crop greater than the CSC is a reduction of fish growth caused by insufficient food supply. Simulation of growth-biomass relationships and how they are influenced by stocking/cropping strategies is possible by modification of the bioenergetics model described above.

Water quality maintenance: Management of pond water quality to ensure optimum (cost-effective) water quality (primarily adequate DO and low UIA levels) is yet another means of enhancing fish yields. Use of this strategy requires knowledge about water quality dynamics in relation to environmental variables and management strategies. Models capable of describing the dynamics of some water quality parameters (e.g., temperature, DO) have been developed by the UCD-DAST. These models have primarily focused on short-term simulation of water quality. For pond management and planning, predictive models are much more useful. Therefore, it would be desirable to modify existing water quality models into long-term simulation tools. It would also be valuable to simulate the effects of different management strategies (e.g., depth management, fertilization, feeding, stocking density) on water quality. This can be accomplished by integrating models of water quality and bioenergetics (described above).

Development of a fish bioenergetics model applicable to pond aquaculture and its integration with water quality models is crucial to evaluation of different management practices because it provides a quantitative means of assessing the effects of manipulating the pond environment on fish yield, which is ultimately the yardstick by which any management practice is judged. Improved understanding of the dynamics of fish growth and water quality, and identification of future research needs is also anticipated from the results of this study.

Methods: The OSU-DAST is currently developing a simulation model for fish growth based on earlier work by other DAST members, which will be refined to describe fish (specifically tilapia) energetics in ponds. The generalized energetics model of Ursin (1967) will form the basis for a mechanistic analysis of the effects of different variables including food availability (or feeding level), stocking density, fish weight, temperature, DO, UIA and spawning on fish growth. The food availability function will include natural food and supplementary feed components, which in turn are determined by fertilization and feeding practices. Functions that describe temperature, DO, UIA and size effects on fish growth in current bioenergetics models (e.g., Brett 1979; Cuenco et al. 1985) will be calibrated for tilapia growth based on CRSP data and information reported in the literature.

Existing models of water quality developed by the UCD-DAST (which may include Total Aquaculture Pond or TAP, temperature and DO stratification) will be modified to enable long-term simulations to be conducted. These models will be linked to the bioenergetics model so that values for water quality variables that affect fish growth will be directly available for simulating fish growth. Management factors that influence both water quality and fish growth will be part of the integrated water quality/fish bioenergetics model. The intent is to develop a simulation tool that can be used to examine the effects of a variety of management practices and environmental variables on fish growth.

Schedule: An initial version of the fish bioenergetics model will be developed by April, 1994. Modification of water quality models and formal linkage with fish bioenergetics will be accomplished by January, 1995.

DAST Study 5: Design and Implementation of the Decision Support System

Objectives:

1. Develop a software environment suitable for the DSS.
2. Transfer current algorithms in PONDCLASS to the new environment.
3. Implement techniques of economic analyses in the DSS.
4. Develop capabilities for decision making and optimization in the DSS.

Significance: Recommendations for management of pond aquaculture facilities must consider both the response of fish to changes in the pond environment caused by management strategies, and the economic implications of such strategies. The DAST studies described are tools that can be used to examine biological responses of ponds to different management strategies. Such tools can be combined with traditional techniques of economic analyses to provide a bioeconomic approach for planning and managing ponds. Economic techniques to analyze the fish production process range from simple enterprise budgets to more complex dynamic simulation methods (Allen et al. 1983). Enterprise budgets tend to view the production process as a static one whereas simulation methods based on dynamic models of water quality and fish bioenergetics can be used to describe output behavior over a range of environmental and management conditions. It may also be possible to couple expert systems with simulation methods (Ernst et al. 1993) and attempt to optimize production according to specified economic goals or criteria such as least cost or maximum profit.

The present version of the DSS developed by the DAST is restricted to providing guidance for pond fertilization, and the software (PONDCLASS) has limited scope for expansion beyond this domain. Integration of concepts of expert systems with a simulation environment for water quality, fish bioenergetics and economic models requires a much more sophisticated software architecture. Besides support for bioeconomic analysis and planning of a pond aquaculture facility (ranging from one or small ponds with minimal inputs to more intensely managed medium or large scale farms), the DSS is intended to be used as a tool for managing ponds. Thus, two general levels of information may be required: (i) dynamics of individual ponds from a biological response point of view, and (ii) facility level (single to multiple pond) management and economic assessment. This can be achieved using object oriented programming environments which support the capability of both analyzing single objects in depth (e.g., the dynamics of water quality and fish bioenergetics in one pond) and linking several objects for simultaneous analysis (e.g., multiple ponds for facility level management and economic assessment). It will therefore be necessary to examine different object oriented programming environments, and design one suited to the DSS.

The DSS will represent a first step towards an integrated approach that synthesizes water quality, fish bioenergetics and economic aspects of pond fish production into a common format that can be used by planners, managers, and researchers to explore a wide variety of production alternatives such as fertilization, supplementary feeding, stocking and cropping strategies, and water quality maintenance. Inclusion of expert system components (i.e., economic criteria and decision making capabilities) will provide guidance for users to plan or operate facilities at optimal levels of fish production.

Methods: Software development will involve adaptation of an existing object oriented DSS framework (Ernst et al. 1993) that provides capabilities for decision making relevant to finfish aquaculture (specifically salmonid production) for use in pond management. This framework combines simulation and expert systems approaches to allow diagnosis of potential management-related problems and provide recommendations for management strategies. The software architecture will be modified to enable examination of the dynamics of individual ponds and facility level analysis using object oriented programming methods.

It will be necessary to convert existing PONDCLASS code to the object oriented environment so that fertilizer recommendations can be generated. In the initial phase of DSS development, simple capabilities for economic analysis based on enterprise budgets will be supported. Previous CRSP work on enterprise budgets (Engle and Skladany 1992; Hanson et al. 1992) will be used to identify fixed and variable cost factors that should be included in this kind of analysis.

The NPP, fish bioenergetics and water quality models outlined in Study 3 and 4 above will be programmed in the environment so as to allow smooth integration into the DSS. These components will be added to the overall DSS together with economic optimization criteria and decision making capabilities (in the form of decision rules for optimal pond production). Economic optimization criteria will be based on a synthesis of published information on methods that have been used to analyze the economics of aquaculture production facilities. The methods used may take the form of traditional production/cost functions (Amir and Knipscheer 1989), simulation techniques (Allen et al. 1983), heuristic methods that result in decision rules (Leung and Shang 1989) or a combination of different techniques.

Schedule: Software architecture development suited to the DSS and conversion of existing PONDCLASS code will be accomplished by December 1993. Development of the framework for enterprise budget analysis and its implementation will be a simultaneous activity through December 1993. The initial version of the DSS will be organized with preliminary documentation, and delivered to CRSP participants for internal review by 30 April 1994.

Feedback from the review process of the first version will be incorporated during July-August 1994. Inclusion of economic optimization criteria and decision making capabilities in the second version of the DSS will be accomplished by January 1995. Testing, refinement and validation of the DSS will be a simultaneous activity through January 1995. The overall DSS will be organized and delivered with a draft of the supporting documentation to the CRSP for review by 30 April 1995. Final refinement of the DSS based on comments by the reviewers and complete documentation (including models used in the software and the design philosophy) will be accomplished by August 1995.

DAST Study 6: Global Experiment (All Projects)

Objectives:

1. Generate quantitative baseline information on biological and physico-chemical parameters of ponds treated identically at all CRSP sites.
2. Field test current fertilization guidelines of the CRSP at all sites.

Significance: One of the primary goals of the PD/A CRSP is to conduct common experiments at the different field sites to facilitate comparison of pond dynamics and production among sites. Such experiments are critical to enhance understanding of pond ecosystems, develop better management practices and improve models of pond dynamics (specifically water quality and fish yield models). Fertilization guidelines that are applicable to sites across a wide variety of water, soil, and climate characteristics have recently been developed by the DAST and implemented in the form of a decision support system called PONDCLASS (Lannan 1993). Preliminary field testing of the guidelines in Thailand and the Philippines has given satisfactory results. A more thorough testing of PONDCLASS at all the CRSP sites is to be undertaken in this study. The assumptions that will specifically be examined in this phase of testing include the concept of fixed fertilizer application rates of nitrogen (N) and phosphorus (P) versus fertilizer application based on anticipated pond productivity and background nutrient concentrations, lime addition to neutralize soil acidity, enhancement of alkalinity by liming in potentially carbon limited ponds, and effects of nutrient availability from manures on the efficiency of fertilization practices. Economic information on fertilizer costs will also be collected to enable comparison with traditional fertilization practices.

The quantitative information from the common treatment to be conducted at all sites will allow comparison among the CRSP sites, and will provide valuable water, soil, and climate data for refinement of water quality/fish yield models. Sufficient feedback on a number of assumptions that have been used to develop present CRSP fertilization guidelines will be generated to allow further improvement of the guidelines.

Experimental Design: A minimum of two treatments (3-4 replicates per treatment) will be conducted at all CRSP sites. The required treatments are (i) fixed fertilizer application (ii) fertilizer application according to PONDCLASS recommendations. The first treatment involves application of 250 kg/ha of poultry manure/ha/week (dry matter basis) supplemented with urea and TSP to provide a nitrogen supply of 28 kg/ha/week at an N:P ratio of 4:1. Calculations of N and P for this treatment will be based on the total N and P in the fertilizers, not the proportion expected to become available. For the second treatment, each of the replicate ponds will be managed independently using PONDCLASS as per the specific instructions discussed below.

Fertilizers will be applied every week, on the day following water analyses and updating PONDCLASS fertilization guidelines. Manure samples will be analyzed on a regular basis (at least once a month) prior to their application. Costs and amounts of fertilizers and lime used for each of the treatments will be recorded for the entire duration of the experiment.

Data collection: Standard data collection protocol as outlined in the Sixth Work Plan.

Duration: 150 days.

Fish Stocking Rate: 2 fish/m^2 (male *Oreochromis niloticus*).

Egypt Project

Grant period: 1 October 1992 through 30 September 1994

Cooperating Institutions:

Central Laboratory for Aquaculture Research, Abbassa
Director

Auburn University
Dr. Claude E. Boyd
Dr. Bryan L. Duncan
Dr. Bartholomew W. Green

University of Oklahoma
Dr. William Shelton

University of Hawaii
Dr. E. Gordon Grau
Dr. Kevin Hopkins

Oregon State University
Dr. Martin Fitzpatrick
Dr. Carl Schreck

University of Michigan/
Asian Institute of Technology, Thailand
Dr. James Diana
Dr. C. Kwei Lin

Introduction:

While aquaculture has been practiced in Egypt for thousands of years, currently reported fish yields from aquaculture ponds are low. Systematic aquacultural research in Egypt was initiated in the mid-1980's upon inauguration of the Central Laboratory for Aquaculture Research (CLAR). The Egypt Pond Dynamics/Aquaculture collaborative Research Support Program (PD/A CRSP) research effort, which will complement previous and concurrent CLAR research efforts, will concentrate in four areas: the Global Experiment, bioconversion, polyculture and biotechnology.

Results of PD/A CRSP Global Experiment in other countries have identified a number of tilapia pond management strategies that have high fish yields and positive economic returns. These pond management systems were developed in countries located in the tropics, however Egypt is located in the sub-topics and has an arid climate. Year I Global Experiment research in Egypt will test performance of established PD/A CRSP pond management systems under local climatic, edaphic and water quality conditions. Egypt is located in the northern-most part of tilapia's natural range. Occasional, unseasonably-cold winters can kill unprotected stocks of *Oreochromis niloticus* and jeopardize aquacultural operations that rely on this species. *O. aureus*, another indigenous species, is more cold tolerant and may be a substitute for *O. niloticus*. Thus Year II Global Experiment research will compare pond performance of these two species under different nutrient input regimes.

Aquatic plants and snails are abundant in many fish ponds in Egypt and represent a nutrient and energy sink, unutilizable by tilapia for growth. The aim of the bioconversion research is to select fish species that can convert the immobilized nutrients and energy in plants and snails into fish flesh. Grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) are the species identified to bioconvert the plant and snail biomasses, respectively.

The bioconversion studies are not an end unto themselves, but are a base upon which to build a more complex polyculture system. Polyculture studies will combine the grass carp-black carp base stocking with different native aquacultured species alone and in combination to examine interactions among species and in an attempt to produce higher fish yields.

The early age at which tilapia attain sexual maturity and their ability to spawn repeatedly during a culture cycle have been serious impediments in the development of successful tilapia production systems. Stocking of all-male fish has proven itself successful in mitigating the negative impact on yield of marketable fish of high numbers of produced offspring. Production of tilapia fingerling populations comprised of greater than 95% males has been routinely accomplished by fish farmers through hormonal sex reversal, but is presently not approved for this use in animals. However, the approval process with FDA has been initiated and biotechnology research will involve participation in clinical field trials to collect data to support the new animal drug application. In addition, biotechnology research will investigate other modes of hormone action, physiological aspects of methyltestosterone treatment, production of "YY" supermale tilapia in order to reduce use of androgen treatment, and development of methods for cryopreservation of tilapia sperm for use in breeding programs.

WORK PLAN FOR COLLABORATIVE RESEARCH IN POND DYNAMICS/AQUACULTURE IN EGYPT

Grant no. 263-0152-G-00-2231-00
(Period of Performance: 1 October 1993 to 30 September 1994)

	data collection (analysis) period	report due
1. Global Experiment		
Study A. Validation of PD/A CRSP pond management strategies	(7/93-11/93)	9/94
Study B. Yield characteristics of two species of tilapia under different pond environments	(5/94-8/94)	9/94
2. Bioconversion		
Study A. Grass carp	(3/93-12/93)	4/94
Study B. Black carp	(3/93-12/93)	4/94
Study C1. Grass carp/black carp	(3/93-12/93)	4/94
Study C2. Grass carp/black carp (feed)	(3/94-8/94)	9/94
Study D. Tilapia/clarias	(7/93-10/93)	4/94
3. Polyculture		
Study A & B. Grass carp/black carp plus tilapia/clarias	(3/94-8/94)	9/94
Study C. Grass carp/black carp plus mullet	(3/94-8/94)	9/94
Study D. Grass carp/black carp plus tilapia/clarias plus mullet	(3/94-8/94)	9/94
4. Biotechnology		
Study A1. Progeny testing to identify YY male tilapia	(5/93-7/94)	9/94
Study A2. Use of 17-alpha methyltestosterone for tilapia sex reversal	(9/93-7/94)	9/94
Study B1. 17-alpha methyltestosterone hatchery trials	(1/93-9/93)	1/94
Study B2. 17-alpha methyltestosterone pond trials	(10/93-6/94)	9/94
Study C1. Characterization of androgen receptors in testes of tilapia	(10/92-7/93)	1/94
Study C2. Masculinization of tilapia	(6/93-6/94)	9/94
Study C3. Generation of tilapia that sire only male offspring	(6/93-6/94)	9/94
Study C4. Cryopreservation of tilapia sperm	(1/94-6/94)	9/94

Egypt Study 1: Global Experiment

Egypt Study 1A. Validation of PD/A CRSP pond management strategies.

Objective: Quantify tilapia yields and production economics of established PD/A CRSP pond nutrient input strategies under climatic, edaphic and water quality conditions found in Egypt. Compare yields obtained with traditional Egyptian management practices to those obtained with PD/A CRSP production practices.

Significance: PD/A CRSP researchers have developed a number of pond management practices that result in high yields of tilapia and that generate profit for the fish farmer. These production systems, which were developed in the tropics, have not been tested in a subtropical/temperate climate. Current pond management practices for tilapia production in Egypt are based on low rates of fertilization combined with a low-quality supplemental feed, and fish yields are low. Testing of PD/A CRSP management practices will determine whether these technologies are applicable in the subtropics. Economic analyses will determine whether these systems are economically feasible in Egypt.

Null Hypotheses: Primary productivity and tilapia yields will not differ with management system. Economic viability of tilapia production will not differ with management system.

Experimental Design: Completely randomized design with five treatments and four replicates per treatment:

"Traditional" Egyptian system	Chicken litter: initial application of 300 kg/feddan, followed by monthly applications of 100 kg/feddan. Triple superphosphate applied at 30 kg/feddan every two weeks. Urea applied at 10 kg/feddan every two weeks. Mixed-sex tilapia stocked. Commercial fish ration (25% protein) only fed daily at 3% of fish biomass.
"Enhanced" Egyptian system	Chicken litter only applied at 1,000 kg/ha per week for the first eight weeks. Commercial fish ration (25% protein) only fed daily at 3% of fish biomass beginning on day 61. Mixed-sex tilapia stocked.
Feed only	Commercial fish ration (25% protein) only fed daily at 3% of fish biomass. Monosex tilapia stocked.
Fertilization then feed	Chicken litter only applied at 1,000 kg/ha per week for the first eight weeks. Commercial fish ration (25% protein) only fed daily at 3% of fish biomass beginning on day 61. Monosex tilapia stocked.
Chemical fertilization	Chemical fertilization at N:P of 4:1. Nitrogen, as urea, added at 25 kg N/ha per week. Phosphorus, as triple superphosphate, added at 14.3 kg/ha per week. Monosex tilapia stocked.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, twenty ponds, 0.1 ha each, approximately 1 m deep.

Culture Period: 150 days.

Fish Stocking Rate: Oreochromis niloticus fingerlings stocked at 20,000/ha; all-male or mixed-sex fingerlings will be used as indicated above. Fingerling *Clarias gariepinus* will be stocked into all ponds at 2,500/ha about two months after tilapia.

Nutrient Inputs: Chicken litter, nitrogen as urea, and phosphorus as superphosphate all applied at the above-indicated rates. Feed will be offered at 3% of fish biomass six days per week.

Water Management: Replace evaporation and seepage losses periodically.

Sampling Schedule: Standard protocol except as noted:
Water quality:
- Chlorophyll *a*: 1 sample/wk.
- Primary productivity: 1 sample/2wk.
- Chemical analyses: 1 sample/wk.

Statistical Methods: ANOVA.

Schedule: Data collection, July 1993 - November 1993; technical report, September 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. Claude Boyd/Auburn University
Dr. Bryan Duncan/Auburn University
Dr. Bartholomew W. Green/Auburn University

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 1B. Yield characteristics of two species of tilapia under different pond environments.

Objectives: Compare production characteristics and production economics of *Oreochromis niloticus* and *O. aureus* reared in ponds managed under two different nutrient input regimes.

Significance: PD/A CRSP research designs heretofore were based on use of *O. niloticus* as the test species. Assurance of adequate *O. niloticus* fingerling availability in Egypt requires indoor (greenhouse) over-wintering facilities for fingerlings and brood fish. Severe cold weather during the winter of 1991/92 decimated unprotected stocks of *O. niloticus* in the delta region of Egypt: to date, stocks do not appear to have recovered. *O. aureus*, much more tolerant of cold temperatures, survive winters in Egypt without having to be over-wintered indoors. In addition, production ponds may be able to be stocked earlier in the season with *O. aureus* than with *O. niloticus*. Similar production characteristics for both species under both pond nutrient management regimes will provide data to support selection of one species as the more appropriate tilapia species to culture in the delta region of Egypt.

Null Hypotheses: Tilapia yields will not differ with species nor with pond management system. There will be no significant interaction between species and nutrient input regime. Economic viability of tilapia production will not differ with species nor with pond management system.

Experimental Design: Completely randomized design in 2 x 2 factorial arrangement, where the factors are tilapia species (*O. niloticus* and *O. aureus*) and pond nutrient input regime (fertilization versus feed); there will be 4 replicates per treatment. The fertilization regime only involves application of fertilizer. In the second treatment, chicken litter will be applied to ponds at 1,000 kg/ha weekly for the fist 8 weeks followed by feed only.

Pond Facilities: Central Laboratory for Aquaculture Research Abbassa, twenty ponds, 0.1 ha each, approximately 1 m deep.

Culture Period: 150 to 180 days.

Fish Stocking Rate: All-male *Oreochromis niloticus* or *Oreochromis aureus* fingerlings stocked at 20,000/ha. Fingerling *Clarias gariepinus* will be stocked into all ponds at 2,500/ha about two months after tilapia are stocked.

Nutrient Inputs: Nitrogen, as urea, and potassium nitrate, applied at 25 kg/ha per week, and phosphorus, as superphosphate, applied to maintain N:P of 4:1. Feed will be offered at 3% of fish biomass six days per week.

Water Management: Replace evaporation and seepage losses periodically.

Sampling Schedule: Standard protocol except as noted:
Water quality:

- Chlorophyll *a:* 1 sample/wk.
- Primary productivity: 1 sample/2wk.
- Chemical analyses: 1 sample/wk.

Statistical Methods: ANOVA.

Schedule: Data collection, May 1994 - August 1994; technical report, September 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. Claude Boyd/Auburn University
Dr. Bryan Duncan/Auburn University
Dr. Bartholomew W. Green/Auburn University

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 2: Bioconversion

Egypt Study 2A. Grass carp.

Objective: Evaluate grass carp production based on autochothonous aquatic plants.

Significance: Studies proposed within the bioconversion component are designed to utilize the stored energy of abundant resources in the Egyptian aquatic culture units. Currently aquatic plants are a nuisance and a tremendous energy sink in most fish ponds. Bioconversion of plants by fish into usable protein, will simultaneously reduce the need for mechanical plant control. Bioconversion is not an end in itself, but can be viewed as a building block for more complex polyculture systems. The grass carp will be used in the bioconversion of plants. Concomitant studies will test black carp and the African catfish in utilization of snails and tilapia young, respectively.

Null Hypothesis: Fish growth will not be affected by stocking rate: final plant biomass will not be affected by grass carp stocking.

Experimental Design: Two stocking rates (high and low) with six replicates plus a series of control ponds, none will include outside nutrient input.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, fifteen 1 feddan (ca. 4,000 m^2) ponds (includes 3 controls).

Culture Period: 150 days.

Fish Stocking Rate: Grass carp of 500 g average weight stocked at approximately 280/ha and 60/ha.

Nutrient Input: No fertilizer or feed - aquatic plants will be initially cut and quantified.

Water Management: Replace evaporation/seepage losses weekly.

Sampling Schedule: Fish growth - (unable to do periodic sampling due to vegetation). Initial weight will be compared to weight at harvest. Plants - periodic visual coverage of regrowth plus area quadrant sampling. Snails - biomass and species composition through periodic sampling. Cericariae infestation of sampled snails will be included. Control ponds will be evaluated similarly and data from this study will be compared with studies 2B and 2C.

Statistical Method: ANOVA, regression analysis.

Schedule: Data collection, March 1993 - December 1993; technical report, April 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 2B. Black carp.

Objective: Evaluate black carp production from within-pond snail biomass.

Significance: Snail biomass in Egyptian ponds represents a large unutilized energy source; in addition, two species are intermediate hosts for two forms of human bilharzia. The energy sink can be tapped by black carp through their natural food habits. Reduction in the snail population may also reduce the incidence of shed cercariae within the fish culture ponds, thereby reducing risk of body contact.

Null Hypothesis: Fish growth will not be affected by stocking rate; snail biomass will not be affected by fish stocking rate.

Experimental Design: Random design of two stocking rates with three replicates (control of study 2A will provide reference for this study).

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, nine 1 feddan ponds.

Culture Period: 150 days.

Fish Stocking Rate: Black carp of 50 g stocked at 115/ha and 229/ha.

Nutrient Input: No supplemental feed or fertilizer.

Water Management: Replace losses from seepage/evaporation weekly.

Sampling Schedule: As in study 2A.

Statistical Method: ANOVA, regression analysis.

Schedule: Data collection, March 1993 - December 1993; technical report, April 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 2C1. Grass carp/black carp.

Objective: Evaluate grass carp and black carp production from within-pond plant and snail biomass, respectively, and examine potential interaction.

Significance: Refer to Bioconversion Studies 2A and 2B. Communal stocking may affect black carp production since plants should be reduced by the grass carp. This change may reduce the food supply for snails, but also will increase vulnerability to black carp predation.

Null Hypothesis: Grass carp growth will not be affected by density; black carp growth will not be affected by presence of grass carp; snail biomass will not be affected by presence of grass carp.

Experimental Design: Grass carp will be stocked at one level (28/ha) with increasing rates of black carp (from 76 to 240/ha). Size at stocking will be the same as in studies 2A and 2B. Bioconversion Studies 2C will be considered as initiating the polyculture investigation.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, four 1 feddan ponds.

Culture period: 150 days.

Fish Stocking Rate: Grass carp of 500 g average weight will be stocked at 286/ha, while 50 g black carp will be stocked at rates from 76-240/ha.

Nutrient Input: No supplemental feed or fertilizer.

Water Management: Replace losses from seepage/evaporation weekly.

Sampling Schedule: See studies 2A and 2B.

Statistical Method: ANOVA.

Schedule: Data collection, March 1993 - December 1993; technical report, April 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma
Dr. Kevin Hopkins/University of Hawaii

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 2C2. Grass carp/black carp/feed.

Objective: Evaluate grass carp/black carp pond production with and without supplemental feed and in relation to size at stocking.

Significance: Bioconversion Studies merge with polyculture systems as communal stockings increase in complexity. Positive and negative interactions may occur in two-species systems, and may be additionally affected by different nutrient conditions. Natural food habits of grass carp/black carp may change in the presence of supplemental feeding as under polyculture management and in relation to size at stocking.

Null Hypothesis: Fish growth will not be affected by supplemental feeding; plant and snail biomass will not be different under either regime of nutrient input or size of stocked fish.

Experimental Design: Large-size grass carp and large-size black carp will be stocked in six ponds, each at a single stocking rate, but half will receive supplemental feeding. Both species will be stocked in three additional ponds at rates comparable to study 2C1.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, nine 1 feddan ponds.

Culture Period: 180 days - minimum.

Fish Stocking Rate: Grass carp (ca. 2 kg) at 500/ha and black carp (ca. 1 kg) at 100/ha in six ponds; grass carp (ca. 500 g) at 750/ha and black carp (ca. 50 g) at 400/ha in three other ponds.

Nutrient Input: Feeding treatments will receive supplemental diet according to the Egyptian practice.

Water Management: Replace losses from evaporation/seepage weekly.

Sampling Schedule: Fish growth - approximately monthly; plants and snails - as described in Bioconversion Study 2A.

Statistical Method: ANOVA.

Schedule: Data collection, March 1994 - August 1994; technical report, September 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma
Dr. Kevin Hopkins/University of Hawaii

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 2D. Tilapia/clarias.

Objective: Examine interaction of tilapia and Clarias in communal culture.

Significance: Refer to Bioconversion Study 2A. The reproduction of tilapia within culture systems is detrimental to the quality of yield, but represents an energy source utilizable by predator fishes. The African catfish will be evaluated in tilapia systems as a bioconverter of unwanted tilapia offspring, which will reduce this management problem while providing a more usable crop.

Null Hypothesis: Clarias will not utilize tilapia young as food.

Experimental Design: Clarias will be stocked at a single rate to verify published information under local conditions. A single stocking rate in four replicates with the Nile tilapia (hormone monosexed) will be compared with a treatment of mixed-sex stocked population using a complete randomized design. See Global Experiment Study 1A for more discussion.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, four 1,000 m^2 ponds within the Global Experiment Studies.

Culture Period: 120 days.

Fish Stocking Rate: Tilapia at $2/m^2$; Clarias at 2,500/ha.

Nutrient Input: Feed (25% protein) daily at 3% tilapia body weight.

Water Management: Replace losses from seepage/evaporation weekly.

Sampling Schedule: Fish growth - monthly; water quality and other parameters according to the protocol of the Global Experiment.

Statistical Method: T-test.

Schedule: Data collection, July 1993 to October 1993; technical report, April 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma
Dr. C. Kwei Lin/Asian Institute of Technology

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 3: Polyculture

Egypt Study 3A and 3B. Grass carp/black carp plus tilapia/clarias.

Objective: Test the basic combination of grass carp/black carp with the addition of tilapia/ Clarias.

Significance: Yields in monoculture vary with the species selected; combination of several species potentially increases yields if there are no negative interactions. However, for maximum benefit, some synergism is desired so that production is complemented and overall yield is greater than just the sum of the component species productions.

Null Hypothesis: Species combinations will result in no greater production than the sum of individual species yields grown separately.

Experimental Design: The foundation of the basic stocking of the Chinese carps (grass and black carp) as determined in the Bioconversion Studies will be combined with tilapia/ clarias, stocked in triplicate.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, six 1 feddan ponds.

Culture Period: 180 days (minimum for Chinese carps); 120 days (minimum for tilapia/ clarias).

Fish stocking rate: Grass carp/black carp (2 and 1 kg, respectively) at 500/ha and 100/ha respectively; tilapia/clarias (20-25 g) at 2/m^2 and 2,500/ha, respectively.

Nutrient Input: supplemental feed and fertilizer in accordance with the results of the Bioconversion Studies.

Water Management: Replace losses from evaporation/seepage weekly.

Sampling Schedule: As outlined in the Bioconversion Studies.

Statistical Methods: T-tests.

Schedule: Data collection, March 1994 to August 1994; technical report, September 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma
Dr. C. Kwei Lin/Asian Institute of Technology

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 3C. Grass carp/black carp plus mullet.

Objective: Test the basic combination of grass carp/black carp with the addition of mullet.

Significance: Yields in monoculture vary with the species selected; combination of several species potentially increases yields if there are no negative interactions. However, for maximum benefit, some synergism is desired so that production is complemented and overall yield is greater than the sum of the component species productions.

Null Hypothesis: Species combinations will result in no greater production than the sum of individual species yields grown separately.

Experimental Design: The foundation of the basic stocking of the Chinese carps (grass and black carp) as determined in the Bioconversion Studies will be combined with mullet, stocked in triplicate.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, six 1 feddan ponds.

Culture Period: 80 days (minimum for Chinese carps).

Fish stocking rate: Grass carp/black carp (2 and 1 kg, respectively) at 500/100 per hectare, respectively; mullet fingerlings at 2,000/ha.

Nutrient Input: supplemental feed and fertilizer in accordance with the results of the Bioconversion Studies.

Water Management: Replace losses from evaporation/seepage weekly.

Sampling Schedule: As outlined in the Bioconversion Studies.

Statistical Methods: T-tests.

Schedule: Data collection, March 1994 to August 1994; technical report, September 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma
Dr. C. Kwei Lin/Asian Institute of Technology

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 3D. Grass carp/black carp plus tilapia/clarias plus mullet.

Objective: Examine interactions between grass/black carp base stocking and other species (tilapia/Clarias and mullet).

Significance: Increased complexity of stockings may be beneficial or may have differentially adverse yield impacts on component species. Further, with increasing biomass and nutrient inputs, the water quality may deteriorate.

Null Hypothesis: Species combinations will not increase total yields above the sum of those individual species production levels in separate trials.

Experimental Design: Three replicates of the composite stockings tested in Polyculture Studies 3A-C will be evaluated. The results will be compared to the simpler species associations.

Pond Facilities: Central Laboratory for Aquaculture Research, Abbassa, three 1 feddan ponds.

Culture Period: 180 days for Chinese carps; 120 days for tilapia (minimum).

Fish Stocking Rates: All species will be stocked at rates and sizes outlined in Polyculture Studies 3A-C.

Nutrient Input: Feed and fertilizer at rates determined from Bioconversion Studies.

Water Management: Replace losses from evaporation/seepage weekly.

Sampling Schedule: As described for Polyculture Studies 3A-C.

Statistical Methods: T-tests.

Schedule: Data collection, March 1994 to August 1994; technical report, September 1984.

Principal Investigator/Affiliation:

from the U.S.:
Dr. William Shelton/University of Oklahoma

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 4: Biotechnology

Egypt Study 4A1. Progeny testing to identify "YY" male tilapia.

Objective: Identify male tilapia *Oreochromis niloticus* which may produce offspring having a sex ratio highly skewed toward males.

Significance: Uncontrolled tilapia reproduction is a major handicap in culture situations. Chemical control or reproduction by altering the sex ratio is possible, but often may not be socially acceptable. Biological control through breeding is an alternative approach. Sex determination in *O. niloticus* may be a simple XX female - XY male system. Previous work at Auburn University has resulted in a population of O. *niloticus* where a portion of the males may have the YY genotype. Crossing such males with normal XX females should result in 100% male offspring.

Null Hypothesis: Sex ratios of selected males crossed with normal females will not differ from 50:50. Spawning success of XY males and YY males will not differ.

Experimental Design: Twenty-five males will be selected and tagged from three or more populations where the sex ratio is approximately 75% male (1 XX female : 2 XY males : 1 YY male). They will be individually paired with normal XX females and allowed to spawn. The offspring will be collected once the yolk sac is absorbed and each spawn cultured separately to a minimum average length of 4 cm. The sex ratio of each spawn will be determined by examining the gonads of a minimum of 100 fish. Male fish whose offspring have highly skewed sex ratios will be retained and spawned again in the 1994 season.

Culture Facilities: Fisheries Research Unit, Auburn University, 75 spawning/rearing hapas (2 m^2); 28 concrete tanks (20 m^3).

Culture Period: Field: 120 days; laboratory: 150 days, 1993-1994.

Statistical Methods: Chi-square.

Schedule: Data collection, May 1993 - July 1994; technical report, September 1994.

Principal Investigator/Affiliation:

Dr. Claude Boyd/Auburn University
Dr. Bryan Duncan/Auburn University
Dr. Bartholomew W. Green/Auburn University

Egypt Study 4A2. Use of 17α-methyltestosterone for tilapia sex reversal: Participation in 1993 clinical field trial under U.S. Food and Drug Administration Investigation New Animal Drug Exemption (INAD 8479 C-002 and C-003).

Objective: Participate in the 1993 clinical field trial under U.S. FDA INAD 8479 C-002 and C-003 to collect efficacy and safety data in support of preparation of a New Animal Drug Application.

Significance: Tilapia producers around the world have depended for more than a decade on sex-reversal technology based on oral administration of 17α-methyltestosterone to newly hatched tilapia fry as the most cost-effective, efficient method of producing male fish for grow out. However, this use of 17α-methyltestosterone was not approved for use in animals. Tilapia is currently being produced commercially in many countries for export to the United States and Europe; the tilapia industry is growing rapidly. Given the concerns for the safety of the human food supply, the U.S. Food and Drug Administration, Auburn University, the American Tilapia Association and Zeigler Brothers, Incorporated, applied for and received an Investigational New Animal Drug (INAD) exemption in order to collect data to support the New Animal Drug Application. One activity contemplated for 1993 is the implementation of a clinical field trial by research institutions and commercial tilapia growers throughout the United States and overseas.

Null Hypothesis: Hormone treatment will not alter the male:female ratio from the expected 1:1 ratio. Hormone treatment will not affect fry growth or survival during the treatment period.

Experimental Design: Newly hatched tilapia fry 9 to 11 mm total length (approximately 7 to 12 days old) will be stocked into hapas and/or fiberglass tanks at 2,000 to 10,000 fry/m^2. Fry will be fed a powdered feed containing 60 mg 17α-methyltestosterone/kg at a daily rate of 100 g of feed per kilogram of fish biomass. Fry in the control treatment will have three replicates. At least two separate trials will be conducted.

Culture Facilities: Central Laboratory for Aquaculture Research, Abbassa, 1.6 mm mesh hapas suspended in earthen ponds and/or fiberglass tanks.

Culture Period: Hormone treatment period is 28 days. 60 to 90 days of nursery growth will be required to obtain fish of appropriate size for sexing.

Fish Stocking Rate: Fry will be stocked into hapas and/or fiberglass tanks at 2,000 to 10,000 fry/m^2 during hormone treatment. Nursery pond stocking rate is 125,000 fish/ha.

Nutrient Inputs: During hormone treatment, fry will be fed 100 g feed/kg fish biomass daily. Daily ration will be divided into at least three meals. Treatment duration is 28 days.

Water Management: Evaporation and seepage losses will be replaced weekly.

Sampling Schedule: Complete harvest of treatment hapas/tanks after 28 days. Once fingerlings attain approximately 5 g size, a random sample of 100 fish per treatment will be analyzed for gonad sex by the acetocarmine gonadal squash technique.

Statistical Method: Chi-square, t-test.

Schedule: Data collection, September 1993 - July 1994; technical report, September 1994.

Principal Investigator/Affiliation:

from the U.S.:
Dr. Bartholomew W. Green/Auburn University

from Egypt:
Director/Central Laboratory for Aquaculture Research, Abbassa

Egypt Study 4B1. 17α-Methyltestosterone hatchery trials.

Objective: Characterize growth-promoting effects and sex-reversal effects of 17α-methyltestosterone (MT) on *Oreochromis mossambicus* and *Oreochromis aureus* under controlled hatchery conditions.

Significance: Our laboratory studies show that using MT as a feed additive significantly increases the growth performance of tilapia, *O. mossambicus*. While our laboratory experiments with *O. mossambicus* have been successful, it is important that our findings be extended to other species and verified under actual aquaculture conditions. This study compares the growth-promoting effects of MT between two species of tilapia, *O. aureus* and *O. mossambicus* under controlled hatchery conditions. Also, since male tilapia tend to grow faster than females, this study separates the growth-promoting effects of MT from its masculinizing effects.

Null Hypothesis: The growth performance of *O. mossambicus* and *O. aureus* will not differ among treatments.

Experimental Design: Four factorial experiments with replicate treatments. The factors are: species, dose level, feeding regime of MT, and sampling dates. Methyltestosterone will be administered orally as a feed additive in graded doses of 0 (control), 1, 10 and 25 mg/kg of feed. Feeding regimes are:

- control treatment, animals fed control feed over the entire experiment;
- continuous treatment, animals fed MT-treated feed over the entire experiment;
- delayed (non-masculinizing) treatment, animals fed control feed for the first three months and then fed MT-treated feed for the remainder of the experiment;
- early (masculinizing) treatment, animals fed MT-treated feed for the first three months and then fed control feed for the remainder of the experiment.

Hatchery Facilities: Facilities are located at the Mariculture and Research Training Center, University of Hawaii. Brood stock animals housed in a 2500 liter spawning tank will provide yolk-sac fry for the experiments. The experiments will be conducted in 700 liter tanks approximately 1 m high by 1 m long by 0.7 m wide.

Culture Period: 270 days.

Fish Stocking Rates: Both *O. mossambicus* and *O aureus* yolk-sac fry of mixed sex will be collected from our brood stock facilities and distributed equally between treatments at 300 to 500 animals per treatment group.

Diet: Animals will be fed commercial feed (Purina Trout Chow) treated with MT. Feed pellet size will range from ground feed to 10 mm to accommodate the size of the animals. Initially, fish will be fed at a rate of 3% of the body weight twice daily (6% total). This will be reduced in all tanks simultaneously to 2% twice daily as food consumption decreases.

Water Management: Constant level, flow-through water replacement system.

Sampling Schedule: Samples will be collected from randomly selected animals:

- fish weight, N=20, 2 times per month, to evaluate growth performance;
- fish length, N=20, 2 times per month, to evaluate growth performance;
- sex determination, N=20, 2 times per month, to evaluate sex reversal;
- blood samples, N=20, 1 time per month, to evaluate residual MT levels;
- muscle samples, N=20, 1 time per month, to evaluate residual MT levels;

- liver samples, N=20, 1 time per month, to evaluate residual MT levels;
- gonad samples, N=20, 1 time per month, to evaluate gonado-somatic index.

Calculated Factors:

- feed conversion efficiency;
- condition factor;
- hepato-somatic index;
- gonado-somatic index.

Statistical Method: Four factorial analysis of variance and regression analysis.

Schedule: Data collection, January 1993 to September 1993; technical report, January 1994.

Principal Investigator/Affiliation:
Dr. E. Gordon Grau/University of Hawaii

Egypt Study 4B2. 17α-Methyltestosterone pond trials.

Objective: Characterize growth-promoting effects and sex-reversal effects of 17α-methyltestosterone (MT) on *Oreochromis mossambicus* and *Oreochromis aureus* under pond aquaculture conditions.

Significance: Our laboratory studies show that MT when fed as a feed additive significantly increase the growth performance of tilapia, *O. mossambicus*. While our laboratory experiments with *O. mossambicus* have been successful, it is important that our finding be extended to other species and verified under actual aquaculture conditions. This study compares the growth-promoting effects of MT between two species of tilapia, *O. aureus* and *O. mossambicus* under pond aquaculture conditions. Also, since male tilapia tend to grow faster than females, this study separates the growth-promoting effects of MT from its masculinizing effects.

Null Hypothesis: The growth performance of *O. mossambicus* and *O. aureus* will not differ among treatments.

Experimental Design: Four factorial experiments with replicate treatments. The factors are: species, dose level, feeding regime of MT, and sampling dates. Methyltestosterone will be administered orally as a feed additive in graded doses of 0 (control), 1, 10 and 25 mg/kg of feed. Feeding regimes are:

- control treatment, animals fed control feed over the entire experiment;
- continuous treatment, animals fed MT-treated feed over the entire experiment;
- delayed (non-masculinizing) treatment, animals fed control feed for the first three months and then fed MT-treated feed for the remainder of the experiment;
- early (masculinizing) treatment, animals fed MT-treated feed for the first three months and then fed control feed for the remainder of the experiment.

Pond Facilities: 1/3 acre, 1 to 1.5m deep, 60 m^2 ponds.

Culture Period: 270 days.

Fish Stocking Rates: O. mossambicus and *O. aureus* fry of mixed sex will be collected from brood stock animals and distributed equally between treatments at 5,000 to 10,000 animals per pond.

Diet: Animals will be fed commercial diet treated with MT. Feed pellet size will range from ground feed to 10 mm to accommodate the size of the animals. Initially, fish will be fed at a rate of 3% of the body weight twice daily (6% total). This will be reduced in all ponds simultaneously to 2% twice daily as food consumption decreases.

Water Management: Constant level with replacement for evaporation and seepage as required.

Sampling Schedule: Samples will be collected from randomly selected animals:

- fish weight, N=20, 2 times per month, to evaluate growth performance;
- fish length, N=20, 2 times per month, to evaluate growth performance;
- sex determination, N=20, 2 times per month, to evaluate sex reversal;
- blood samples, N=20, 1 time per month, to evaluate residual MT levels;
- muscle samples, N=20, 1 time per month, to evaluate residual MT levels;
- liver samples, N=20, 1 time per month, to evaluate residual MT levels;
- gonad samples, N=20, 1 time per month, to evaluate gonado-somatic index;
- pond substrate, flora and fauna, 1 time per month, to evaluate residual MT levels.

Calculated Factors:
- feed conversion efficiency;
- condition factor;
- hepato-somatic index;
- gonado-somatic index.

Statistical Method: Four factorial analysis of variance and regression analysis.

Schedule: Data collection, October 1993 - June 1994; technical report, September 1994.

Principal Investigator/Affiliation:
Dr. E. Gordon Grau/University of Hawaii

Study 4C1. Characterization of androgen receptors in testes of tilapia.

Objectives: Determine androgen binding characteristics of tilapia testicular cytosol.

Significance: Many of the steroids used to control the gender of tilapia are controlled substances that may yield inconsistent results. New steroids that are less hazardous or more effective are always in demand. Usually, testing different steroids for sex inverting potential requires treatment of fish and subsequent culture until the phenotype can be determined; however, it may be possible to rapidly screen a wide variety of potential sex-inverting steroids by developing methods for measuring steroid binding in gonadal tissue.

Hypotheses: Testicular cytosol of tilapia contains an androgen receptor and a number of steroids are capable of displacing mibolerone binding in testicular cytosol.

Experimental Design: An androgen receptor assay will be developed for testicular cytosol. Cytosol will be prepared by ultracentrifugation from gonadal homogenates of tilapia at different reproductive stages. These cytosolic preparations will be tested for binding activity using mibolerone, a synthetic androgen with demonstrated masculinizing capacity in tilapia. Once the existence of specific binding has been established, it will be possible to look at the potency of a wide variety of steroids for displacing mibolerone from the binding site. The most potent displacing steroids will be those that cause the largest decrease in specific binding of mibolerone at the lowest excess levels. Initial efforts will concentrate on steroids with demonstrated masculinization potential; subsequent efforts will screen a wide variety of steroids.

Laboratory Facilities: Oregon State University, one large stock tank for holding 150-200 tilapia, analytical laboratory for performing binding studies.

Statistical Methods: Scatchard Analysis for binding data.

Schedule: Tissue preparation and data collection, October 1992 - October 1993; technical report, January 1994.

Principal Investigator/Affiliation:

Dr. Carl Schreck/Oregon State University
Dr. Martin Fitzpatrick/Oregon State University

Study 4C2. Masculinization of tilapia by immersion in steroids.

Objectives: Optimize steroid immersion procedures for masculinization of tilapia.

Significance: Currently, masculinization of tilapia typically involves feeding a diet mixed with synthetic steroids to fry. This procedure may require unnecessary environmental and worker exposure to steroids, which have possible negative impacts. Development of steroid immersion procedures to masculinize tilapia would allow for limited exposure of animals and workers to steroids, and containment of steroid in a limited, treatable volume.

Hypothesis: Immersion of fry in 17α-methyltestosterone will produce all-male tilapia.

Experimental Design: Immersion of newly hatched fry in 17α-methyltestosterone will be compared with feeding of this steroid for production of males. Immersion doses will be 400 and 1000 μg/Liter of water; feeding doses will be 4 and 40 mg/kg of food for six weeks beginning at the onset of feeding. Optimum treatment will be that which allows maximum production of males. Hormonally masculinized fish will be mated to normal females and the gender of resulting offspring will be determined to demonstrate the viability of XX males.

Laboratory Facilities: Oregon State University, two aquaria containing a total of 4 males and 10 females for production of eggs, smaller tanks for hormone treatment and raising offspring.

Culture Period: 180-365 days.

Statistical Methods: ANOVA for survival, chi-square for sex ratio.

Schedule: Data collection, June 1993 - June 1994; technical report, September 1994.

Principal Investigator/Affiliation:

Dr. Carl Schreck/Oregon State University
Dr. Martin Fitzpatrick/Oregon State University

Egypt Study 4C3. Generation of tilapia that sire only male offspring.

Objective: To produce tilapia males that sire only male offspring when mated to normal females.

Significance: Fish offer a myriad of mechanisms of sex determination, including genetic sex determination (Tave 1986). *Orechromis niloticus* have a female homogametic (XX) system. If nile tilapia are initially feminized with hormones and then mated with normal males, then some of the resulting offspring will be YY "super" males that are capable of siring only male offspring. A simple technique for producing such males would be beneficial for culture of monosexual male tilapia without the use of synthetic androgens.

Hypotheses: Immersion in estrogens is as effective as dietary estrogen treatment for the feminization of tilapia.

Experimental Design: Immersion of newly hatched fry in ethnylestradiol and estradiol will be compared with feeding of these steroids for production of females. Immersion doses will be 400 and 1000 µg/Liter of water; feeding doses will be 4 and 40 mg/kg of food for six weeks beginning at the onset of feeding. Optimum treatment will be that which allows maximum production of females.

Laboratory Facilities: Oregon State University, two aquaria containing a total of 4 males and 10 females for production of eggs, smaller tanks for hormone treatment and raising offspring.

Culture Period: 180-365 days.

Statistical Methods: ANOVA for survival, chi-square for sex ratio.

Schedule: Data collection, June 1993 - June 1994; technical report, September 1994.

Principal Investigator/Affiliation:

Dr. Carl Schreck/Oregon State University

Dr. Martin Fitzpatrick/Oregon State University

Egypt Study 4C4. Cryopreservation of tilapia sperm.

Objective: To develop cryopreservation techniques for tilapia sperm.

Significance: The establishment of a breeding program using broodstock with desired characteristics, such as the capacity to sire monosex offspring, may be enhanced by the development of cryopreservation technology. Such technology would allow for the long-term storage of genetic information that can be utilized in future breeding to attain specific aquaculture goals (e.g. production of all male tilapia). Particular attention will be paid to developing methods for higher temperature storage to increase the utility of cryopreservation in areas of the world where freezing and ultralow freezing capabilities are limited.

Hypotheses: Cryopreserved tilapia milt will fertilize eggs at the same efficacy as fresh milt.

Experimental Design: Tilapia milt will be cryopreserved in solutions commonly used in our laboratory for preserving salmonid milt. Cryopreserved tilapia milt will be stored at -80° C, -20° C, and at 4° C for one to two months, thawed (or warmed to fertilization temperature), and then tested for the capacity to fertilize tilapia eggs. Fertilization capacity will be compared with that of fresh milt. Optimum treatment will be that which results in the highest survival of offspring.

Laboratory Facilities: Oregon State University, analytical laboratory for cryopreservation, two aquaria containing a total of 4 males and 10 females for production of eggs, smaller tanks for raising offspring.

Culture Period: 180 days.

Statistical Methods: ANOVA for survival.

Schedule: Data collection, January 1994 - June 1994; technical report, September 1994.

Principal Investigator/Affiliation:

Dr. Carl Schreck/Oregon State University
Dr. Martin Fitzpatrick/Oregon State University

Appendix

Instructions for PONDCLASS use

Detailed instructions for the operation of PONDCLASS are available in the User's guide (Lannan 1993) and its use for this experiment outlined in the latest issue of the DAST Newsletter (#13, March, 1993). This information is summarized here.

Creating files in PONDCLASS: Separate files will be created for each of the replicate ponds in the treatment (pgs. 19-22 of the User's guide). The DATA option will be used to enter water and soil information. The following data will be entered during file creation:

1) Baseline water quality parameters, namely alkalinity, pH, dissolved inorganic nitrogen (sum of total ammonia, nitrate and nitrite nitrogen) and phosphorus (soluble orthophosphate) for each pond after filling.

2) Soil characteristics (soil type, CEC, soil pH, PBS, and soil density) for each pond before filling. If PBS and soil density data are not available, the typical value for the soil type at the site (pgs. 46-47, 53-56 of User's guide) will be used.

3) Climate data, namely net primary productivity (NPP) and average water temperature for the 12 months of the year. The NPP data entered will be based on values for each month that are in the higher range of the observations at the site from previous experiments. NPP will be entered in terms of g C/m^3/day. If existing measurements are in terms of g O_2/m^3/day, a factor of 0.288 will be used to convert to carbon units assuming that 1/3.47 g C are fixed for each g O_2 produced (Stumm and Morgan, 1981).

Lime requirement: The lime requirement routine under the MANAGER option (pg. 30-31 of the User's guide) will be used for each pond to estimate the quantity of lime required to neutralize soil acidity. Lime will be applied at the time of pond preparation, after filling the pond and prior to the first application of fertilizers. The recommended quantity is based on pure $CaCO_3$, which will be multiplied by the appropriate coefficient to take into account the neutralizing value for the liming material used (e.g., 0.56 for quick lime or 0.92 for agricultural limestone; see pg. 54 of User's guide).

Determining fertilizer requirements: PONDCLASS will be run separately for each pond in the treatment. The MANAGER option will be used for this purpose (Chapter 4, pgs. 23-32 of User's guide). After the appropriate data file is loaded, and month selected to locate the expected NPP and average temperature, the display requirements option will be used to examine alkalinity, N and P needed. Requirements will be based on optimum alkalinity instead of present alkalinity. Some ponds may require alkalinity enhancement if the optimum alkalinity is less than present alkalinity. If this is observed, lime will be added to enhance present alkalinity to the required optimal level. The quantity of lime required will be approximately equal to the difference between the two alkalinities (mg/L or g/m^3 of $CaCO_3$). Sufficient liming material will be added to satisfy this need, taking into account the neutralizing value of the material (see lime requirement section above). The display requirements screen will be examined each week prior to testing fertilizers, to determine whether alkalinity enhancement is required.

The data file for each pond will be updated every week prior to testing fertilizers. Data required for updating fertilizer requirements are temperature, Secchi visibility, DO, alkalinity, dissolved inorganic nitrogen (DIN) and phosphorus (DIP), and current water depth. All the data will be from early morning measurements. All the sites will follow the same protocol of fertilizing ponds the day after measurements are made, because it may not be possible to complete water analysis and pond fertilization on the same day.

Fertilizer needs will be obtained using the TEST FERTILIZERS option. The default BOD requirement will not be changed (pg. 44 of User's guide). The select fertilizers option will be used, with a value of 3 for the number of fertilizers to be tested whenever the program is used. The DATA option will be used to enter fertilizer data. The three fertilizers that will be used are 1) poultry litter 2) urea and 3) TSP. Because PONDCLASS recommends fertilizer application rates on an 'as-used' as opposed to a dry weight basis for manures, all entries for N and P contents, BOD and local costs of the manure will be on an 'as-used' or 'wet weight' basis. If the 5-day BOD value of the manure cannot be measured, the default value (11 for poultry litter) from Table 6.3 of the User's guide (pg . 50) will be used. N and P availability from the manure will be estimated using the procedure described below, and available N and P information entered into PONDCLASS. Thus, if the manure has a total N content of 2.8% wet weight, and N availability is 50%, only 1.4% N will be entered. Similarly, if the total P content is 1.2% and its availability 75%, 0.9% P will be entered. For inorganic fertilizers, the N and P contents as measured at the sites will be entered. If such data are not available, an N content of 45% f or urea, and a P content of 47.5% for TSP will be used (Table 6.8, pg. 53 of User's guide). The display current values option will be used to determine fertilizer recommendations, and calculations will be based on optimum alkalinity.

Nutrient availability from manures: The total N and P content of the manure sample will be determined following the standard protocol at all sites. Sufficient manure to correspond to an application rate of 500 kg/ha/week (wet weight basis) or approximately 50 g/m^3/week to a 1 m deep pond will be added to triplicate plastic containers (10-20L capacity) containing distilled or deionized water. The containers will be covered and placed in the dark for 7 days. The quantities of DIN and DIP that evolve from the manure during this period will be measured following standard methods of nutrient analysis, and the N and P availability expressed as % of total N and P in the original sample (see Knud-Hansen et al. 1991 or Nath and Lannan 1993). If possible, the availability coefficients will be estimated following this protocol whenever manure samples are analyzed during the course of the experiment. A test of this procedure will be conducted prior to commencing the experiment.

Schedule: This study will be conducted at all field sites concurrently with other Work Plan experiments. Exact dates are indicated in each of the Work Plans for the different sites.

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