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Pond Dynamics/Aquaculture Collaborative Research Support Program

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Thirteenth Annual Technical Report

1 September 1994 to 31 August 1995

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This year's Annual Report is dedicated to the continuing struggle of the Rwandan people who are working and hoping for peace and stability. The members of the Pond Dynamics/ Aquaculture Collaborative Research Support Program mourn the loss of the many individuals who worked with our program, and hope for a return to peace.

Contents

I. CRSP Research Program Background 1

The CRSP Global Experiment and Related Activities 1
Data Analysis and Synthesis 3
Special Topics Research 3
CRSP Work Plans 5

II. Research Program Accomplishments 7

A. Global Studies and Activities 7

The Effects of Fertilization on Growth and Production of Nile Tilapia in Rain-Fed Ponds 9
Nitrogen Fertilization in the Presence of Adequate Phosphorus 18
Experimental Evaluation of Lime Requirement Estimators for Global Sites 27
Experimental Evaluation of Lime Requirement Estimators for Global Sites-Isolation Column Experiment 33
Problem Perceptions, Production Practices, and Economic Incentives for Tilapia Producers in Four PD/A CRSP Countries 36
Decision Support for Pond Aquaculture: Simulation Models and Applications 42
Decision Support for Pond Aquaculture: Parameter Estimation for Simulation Models 62
Central Data Base 68

B. Central America 69

Characterization of Shrimp Farm Effluents in Honduras and Chemical Budget of Selected Nutrients 70
Relationships among Stocking Density, Survival and Yield in Ponds Affected by the Taura Syndrome during Wet and Dry Seasons in Honduras 85

C. East Africa 95

Masculinization of Nile Tilapia (*Oreochromis niloticus*) through Immersion in 17α-Methyltestosterone or 17α-Methyldihydrotestosterone 96
Maximum Voluntary Feed Intake and Growth of Nile Tilapia Fry as a Function of Water Temperature 101
Characterization of African Soils and Site Evaluation 103
Reproductive Efficiency of Nile Tilapia (*Oreochromis niloticus*) and "Red" Tilapia (*Oreochromis* spp.) and Comparative Growth and Efficacy of Sex Reversal of Nile Tilapia and "Red" Tilapia 105
Growth and Efficiency of Sex Reversal of Nile Tilapia Fed Hormone Treated Feed Stored Under Different Storage Regimes 108

D. Southeast Asia 109

A Finishing System for Large Tilapia 110
Stocking Density and Supplemental Feeding 117
Diel Cycles of Temperature and Dissolved Oxygen Stratification in Deep Rain-fed Ponds 126
Carbon Dioxide Exchange between Pond Water and the Atmosphere 131
Growth Comparison of Three Strains of Nile Tilapia in Fertilized Ponds 135
Effects of Pond Size 137

E. United States: Data Analysis and Synthesis 141

Aquaculture Pond Modeling for the Analysis of Integrated Aquaculture/Agriculture Systems 142
Stochastic Modeling of Temperature and Dissolved Oxygen in Stratified Fish Ponds 148

F. Special Topics Research 168

Sex Reversal of Tilapia: 17α-Methyltestosterone Dose Rate by Environment, and Efficacy of Bull Testes 168

III. Appendices 171

Appendix A: Acronyms 171
Appendix B: Table of Contents of the 13th Administrative Report 173
Appendix C: Egypt Final Report 177

I. CRSP Research Program Background

The current period was characterized by the following accomplishments: Initiation of a new one-year transition year Work Plan (the Interim Work Plan, 1995-96); completion of research activities scheduled under the Seventh and Interim Work Plans; succesful completion of the Egypt project and publication of research results in the Egypt Project Final Report; revision of the Africa Work Plan and resumption of research activities which were originally planned for Rwanda in Honduras and in the U.S.; and development of site selection criteria for a new African site. The resiliency of the CRSP has enabled it to rebound from the challenges caused by the shadow of the terrible events which engulfed Rwanda.

Management challenges resulted from delays in receiving authorization from USAID for a 5-year program continuation. An interim Work Plan was approved by the Technical Committee in June 95 to cover the one year extension granted by USAID.

The following report summarizes research activities conducted during this transition year. The scientists' descriptions of their research activities have not been edited bu the Management Office except for minor typographical errors.

In all previous years the Management Office devoted extensive efforts to the Annual Technical Reports. Due to an enhanced work load placed on the Management Office from the Continuation proposal and one-year extension, staff were unable to perform this function this year. The companion volume to this Annual Technical Report is the Annual Administrative Report which enumerates achievements in administration, research, and outreach activities, and as well summarizes program history, personnel, financial status, and publications. It also contains the abstracts of all technical reports included in this volume (See Appendix B for the table of contents to the Administrative Report).

The CRSP Global Experiment and Related Activities

Since its inception, the goal of the CRSP has been to improve the efficiency of pond production systems through sustainable aquaculture. The strategy adopted by the CRSP in pursuit of this goal has been to undertake the basic research required to understand and improve the efficiency of pond culture systems.

In 1978, a technical plan proposing this strategy was developed under a planning study funded by USAID. The technical plan reviewed and synthesized literature on state-of-the-art pond aquaculture. Overseas sites were surveyed to determined research needs and availability of local support in host countries. The findings from these surveys were then incorporated into planning guidelines.

The literature overview that was conducted during the planning phase showed that different pond systems exhibited considerable variation in productivity. Pond aquaculture had been practiced for centuries as a highly developed art form, and the literature was replete with reports about practices that had produced high yields. However, the results were often not reproducible when the same practices were applied to other ponds. It was clear that there were subtle differences regulating productivity from pond to pond and from site to site, but the nature of these differences remained obscure.

The Global Experiment was intended as a comparative study of aquaculture pond dynamics—one that would help us begin to understand how and why ponds at different geographic locations function differently, and how the management of those ponds might be adapted to different sets of environmental conditions to optimize production. Hence, a common set of experiments was implemented globally, following a standardized experimental protocol at a number of research sites around the world.

The initial technical design for the Global Experiment involved monitoring environmental and fish production variables at seven geographical locations in six countries (Figure 1). Observations specified in biennial (originally annual) Work Plans are made on twelve or more ponds of similar size at each location. The variables observed, frequency of observation, and materials and methods used are uniform for all locations. Two brackish water and five freshwater research sites were selected in Central America (Panama and Honduras), Africa (Rwanda), and Southeast Asia (Indonesia, Thailand, and the Philippines) in 1982 . All of the sites were within a zone of 15 degrees north and south of the equator and represented the three major tropical regions where advances in pond aquaculture would be most beneficial and most apt to succeed. Subsequent changes in 1987, mainly in response to funding constraints, required that research be continued at only three (Thailand, Rwanda, Honduras) of the six countries originally selected to maintain sites in the three major regions of the tropics. In 1991, the CRSP program was expanded by the initiation of a sub-project in the Philippines (at a new site in Central Luzon) and the beginning of a completely new project in Egypt. Termination of research activities in Panama and funding constraints in 1987 caused a hiatus in brackish water research which was resumed in 1993 with the addition of a new coastal site in Honduras. The outbreak of civil war in Rwanda in April 1994 caused the cessation of all CRSP research activities in Rwanda. The Egypt project, under a separate grant from the Cairo Mission, was originally slated to end in 1994; however, after a positive review, it was extended for half a year and ended in March 1995.

The first cycle of experiments aimed to develop a set of baseline data on ponds at the various sites. Subsequent Global Experiment studies have focused on investigations of the effects of different fertilizer regimes on pond productivity and yield. The Global Experiment has been further strengthened by the addition of the Egypt project (the only arid CRSP site) because researchers can now compare pond processes observed in humid and arid environments. Although all CRSP research activities have ceased in Egypt, the CRSP hopes that collaboration may be resumed in the future. This may happen under the aegis of the International Center for Living Aquatic Resources Management (ICLARM) which is currently considering Egypt as a site for its aquaculture research activities.

As CRSP research progressed through the 1980s, new questions surfaced—questions that differed from site to site and needed to be addressed with specific experiments. This family of experiments, though separate from the standardized Global Experiment, yet performed concurrently with it, is also global in nature. For example, currently all CRSP sites conduct studies on sediment dynamics and their influence on water quality in tilapia or polyculture ponds. Pond soils have been analyzed in an attempt to establish baseline information and to investigate the role of sediments as nutrient sinks. This research dove-tails with the CRSP's interest in the environmental effects of aquaculture on the aquatic environment. The CRSP is currently conducting pioneering research on the influence of pond management practices on effluent load. The aim of this research is to maintain high productivity while preventing nutrient wastage and concurrent pollution. The findings gained from these studies will have world-wide practical application.

After the first few years of Global Experiment research, economic analyses of pond aquaculture systems were added as a component of the aquaculture development strategy in both the U.S. and host countries. Previous research had relied on numerous and often tenuous assumptions that reflected how much remained unknown about the dynamic mechanisms regulating pond productivity and confirmed the inadequacy of the existing database. To find out if contemporary pond management practices were in fact the most efficient, CRSP researchers developed quantitative production functions. An extensive comparison of the socioeconomic dimensions of CRSP production techniques among sites is helping CRSP researchers to understand the similarities and differences of socioeconomic influences on their work.

Data Analysis and Synthesis

CRSP planners recognized at the outset that aquaculture ponds are extremely complex ecosystems. This complexity has been reflected in the number of variables and frequency of observations required by the experimental protocols specified in the CRSP Work Plans. Although researchers at each of the overseas field sites are free to analyze their own data and publish their findings, it was recognized that the management and analysis of the global data set (i.e., the data generated by all the field sites) would require the establishment of a central data storage and retrieval system. This Central Data Base was originally established at Oregon State University and maintained by the Management Entity until Spring of 1993 when it was transferred to the University of Hawaii at Hilo.

Standardized data are tabulated at each research location in accordance with CRSP Work Plans. At the individual sites, data on physical variables (e.g., solar radiation, temperature, and rainfall) and chemical variables (e.g., water and soil characteristics) are collected concurrently with biological measurements (e.g., primary productivity, fish growth, and fish production). Over 160 physical, chemical, and biological variables (approximately 90,000 observations per site and year) are observed. Whereas the resulting sets of data are useful for site-specific studies, the compilation of all the individual data sets into the Central Data Base provides opportunities for many kinds of global analyses. Detailed standardized records such as those found in the CRSP Central Data Base are rare in the aquaculture literature. An internal review commissioned by the Program Management Office confirmed that all data from research activities conducted under the First through the Fourth Work Plans are already in the data base, and entry of data from the Fifth Work Plan is almost completed. The Central Data Base has continued to expand through the inclusion of new data generated under the Sixth and Seventh Work Plans. Other important features of the database are robustness and flexibility which ensure the inclusion of data generated on new sites.

CRSP participants also decided that the comprehensive analysis and interpretation of global data would be greatly enhanced through the formation of an independent team of researchers who could devote their efforts to this type of analysis. This task force was formally established in 1986 as the Data Analysis and Synthesis Team (DAST). The charge of the DAST is to systematically analyze pond processes and to develop computer models that reflect our growing understanding of pond systems. The DAST members are more than end-users of the data base; rather, they participate actively in the design process of the next cycle of Global Experiments. Communication between the DAST and field researchers assures that the experimental design encompasses the information needs of the DAST. The benefits of analyzing global data and synthesizing information into computer models that simulate pond conditions occur on several levels: production management, design, and planning. The quantification of relationships between variables and the effects of different treatments allows farmers to adapt general management techniques to the specific local constraints of climate, water, feed, and fertilizer availability in order to optimize production. The design of production systems will be improved by matching production facilities and costs with production goals.

Special Topics Research

The Special Topics component of the CRSP was created to provide opportunities for host country and U.S. researchers to collaborate on original research directed toward the needs and priorities of each host country. The intent is to strengthen linkages and contribute to the development of research capabilities within host country institutions by providing opportunities for scholarly involvement of faculty and advanced students. This component also provides host country institutions and agencies with access to the human resources of the CRSP in seeking solutions to short-term local problems. Projects focus on specific aspects of the Global Experiment that would benefit from site-specific, detailed investigations.

Proposals for these Special Topics Research Projects are developed collaboratively by the host country and U.S. scientists. The proposals are endorsed by the host country institution and are reviewed by the CRSP Technical Committee and other CRSP advisory groups for technical merit and relevance to the general goals of the CRSP. The projects must also be consistent with USAID and host country development strategies and priorities.

PD/A CRSP Research Locations around the World

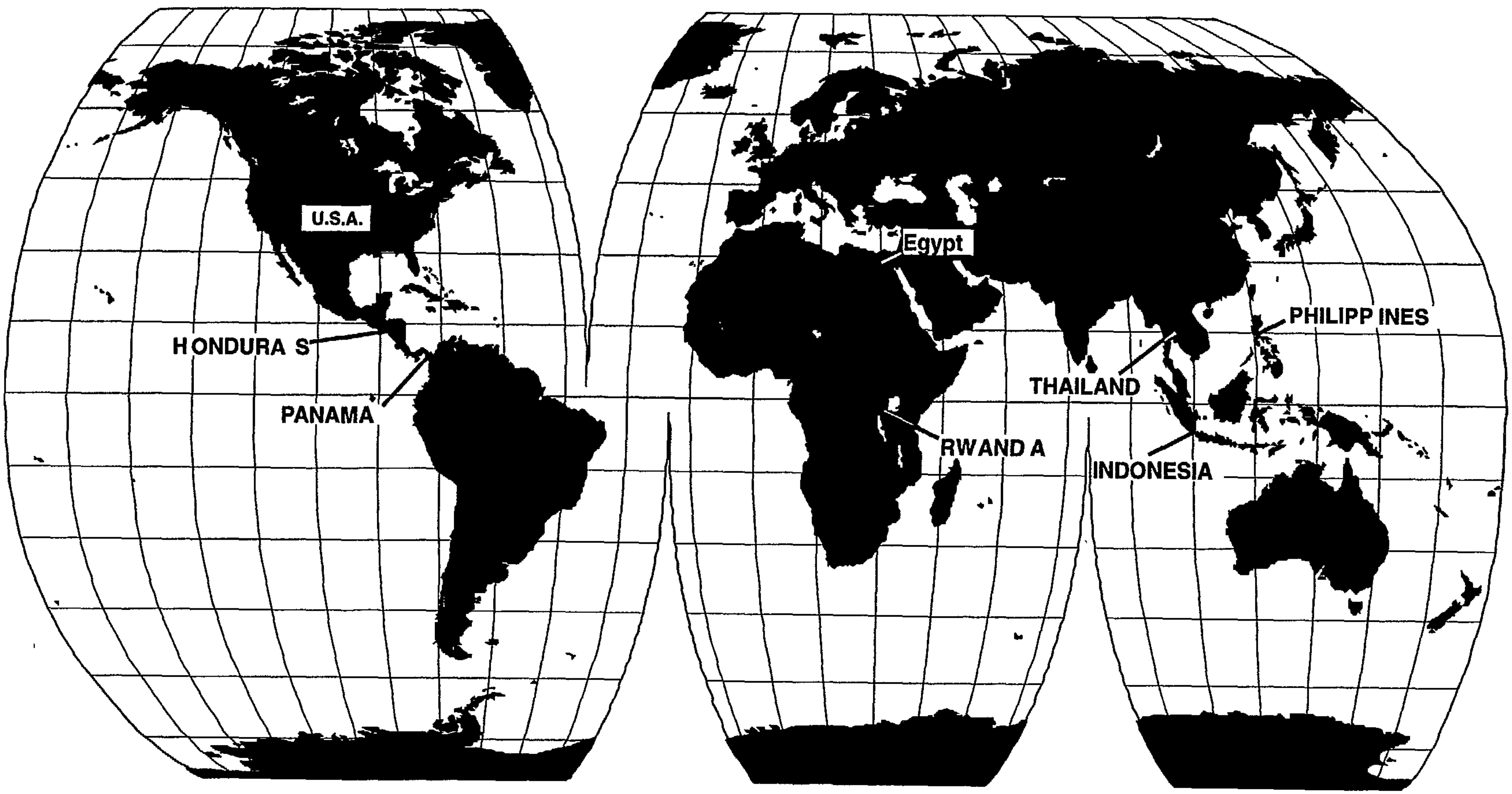

Figure 1. Past and present PD/A CRSP research locations in Central America (Honduras, Panama), Africa (Egypt, Rwanda), Southeast Asia (Indonesia, the Philippines, and Thailand), and the USA.

Although Special Topics Research Projects are an important part of the CRSP, they are not a major component in terms of funding support or time expenditure. Twenty to twenty-five percent of each researcher's time typically is devoted to this activity. The CRSP places high priority on long-term basic research. Host country institutions and USAID Missions, however, often consider basic research activities such as the Global Experiment to be of low priority. Consequently, administrators in the host countries sometimes have difficulty justifying participation in the CRSP. The CRSP support for the Special Topics Research activities helps them to see the value of their institutions' participation in the CRSP.

CRSP Work Plans

From the CRSP's beginning, the Technical Committee of the PD/A CRSP has had the responsibility for developing technical plans to guide the research efforts of each experimental cycle. During the first three cycles of the program, when global experiments were the main emphasis, CRSP Work Plans were developed annually. The First Work Plan specified a standard protocol for the preparation and stocking of ponds at all locations. Research in the Second Work Plan compared the responses of ponds receiving organic fertilizers with the responses of ponds that received inorganic fertilizers. Experiments described in the Third Work Plan investigated the effects of varying levels of organic fertilizers on pond dynamics.

In response to recommendations of the External Evaluation Panel during the first Triennial Review, a biennial approach to Work Plan development and execution was adopted beginning with the Fourth Work Plan. Two-year operating cycles allow more time for completion and evaluation of experiments before plans for the next cycle must be completed.

Although the research program has evolved so that the Global Experiment and site-specific experiments are conducted at the various sites, the concept of a standard protocol for research at all sites has been maintained. The standard protocol was initially introduced as a part of the First Work Plan and has been improved with each subsequent Work Plan. In 1992 it finally evolved into the PD/A CRSP's *Handbook of Analytical Methods*, compiled by the Materials and Method Subcommittee of the Technical Committee and distributed to CRSP participants.

The Fourth Work Plan included tests of specific hypotheses formulated after review of the first three cycles of CRSP research. Special attention was paid to the economic aspects of CRSP pond management procedures. Further, the Data Analysis and Synthesis Team (DAST) started to systematically use the Central Data Base.

The Fifth Work Plan was developed by the Technical Committee in May 1989, and encompassed research efforts carried out between 1 September 1989 and 31 August 1991. In addition to the Global Experiment, each site proposed various studies that addressed specific aquaculture needs of the host countries. Field experiments with farmer-cooperators were initiated, allowing researchers to evaluate their strategies under 'real life' conditions, and strengthening the linkage between research and practice. Economic analysis became another tool by which the CRSP measured the quality of its research achievements. The DAST's efforts focused on refining models and developing fertilizer guidelines.

The Sixth Work Plan, which began on 1 September 1991 and ended 31 August 1993, was approved at the Ninth CRSP Annual Meeting in May of 1991. A 20% funding increase allowed the CRSP to broaden its research scope. Nine supplemental projects were included in the Sixth Work Plan. One of these studies was a preliminary investigation of women's participation in fish culture activities in Rwanda. This study was used to attract a buy-in from USAID's Women In Development program (WID) to perform more complete investigations on the role of gender in fish culture in Rwanda. Also, under the auspices of the Thailand team, research activities were re-initiated in the Philippines.

The Seventh Work Plan is characterized by several changes. The CRSP resumed its original investigation of pond dynamics in brackish water systems, a line of research that had been temporarily suspended when the CRSP's brackish water sites in Panama and the Philippines were lost in 1987. This Work Plan also introduced a new research focus, biotechnology, which has the potential to greatly aid the aquaculture industries in the U.S. and host countries. Experiments originally scheduled to be conducted in Rwanda had to be reassigned to different sites after the outbreak of civil war. It is a sign of the CRSP's resiliency and the global nature of the program that the Africa team was able to regroup and develop a revised Seventh Work Plan whose experiments are currently conducted in Honduras and the United States. Furthermore, research on the influence of elevation on tilapia production originally conducted in Rwanda is now being continued in the Philippines.

The Interim Work Plan covers experiments to be conducted during the transition year (May 1995 through April 1996). This deviation from the usual biennial Work Plan format was necessitated by delays imposed by USAID in the grant renewal process. In addition to a description of experiments, this Work Plan also outlines activities of the Africa Site Selection Team.

This report covers the second year of the Seventh Work Plan for all sites but Egypt. The Egypt project activities were reported separately in the Egypt Project Final Report, which is available as an addendum to this report (Appendix C) beginning on page 177). Studies from Sixth Work Plan and activities initiated under the Interim Work Plan are also included in this report.

II. Research Program Accomplishments

Major accomplishments during the current reporting period include the completion of a number of the activities scheduled under the Sixth and Seventh Work Plans. These include further refinements to several CRSP aquaculture pond models, improvements to the *POND*© decision support system, continued environmental monitoring, and resumption at other CRSP sites of studies which were originally scheduled for Rwanda. Activities described in the Interim Work Plan were begun during this reporting period. As always, efforts to disseminate research results continued through a variety of channels.

Global Studies and Activities

The centerpiece of PD/A CRSP research is the Global Experiment. The Interim Work Plan's Global Experiment attempts to quantify the effect of a particular pond management strategy on water quality and sediment quality by developing nitrogen and phosphorus budgets. Little information is available on the effect of semi-intensive pond management strategies on the quality of pond effluents. Discharge of nutrient-rich pond water may cause deteriorated quality of receiving waters. Development of nutrient budgets will permit researchers to quantify the potential pollution impact of a specific pond management strategy. This experiment is presently being conducted at the Honduras and Thailand sites and results are not yet available.

The following studies also exemplify the global significance of PD/A CRSP research efforts: the investigations of fertilizer effects on growth and production in Thailand and Honduras, the investigations of lime requirement estimators, and the CRSP global socioeconomic research. The CRSP Central Data Base-the world's largest standardized aquaculture database-continues to grow.

Researchers evaluated fertilization strategies for rain-fed ponds in Thailand based on strategies developed for ponds that receive regular water inputs. Regular pond fertilization resulted in the highest fish growth rates. Irregular fertilization yielded lower growth, and fertilizing only at time of stocking yielded the lowest growth. Results of this study will impact farmers in northeast Thailand, whose ponds are typically rain-fed, and who have lacked research-based information on appropriate fertilization regimes.

Researchers in Honduras studied the effects of nitrogen fertilization on water quality and tilapia yield in ponds supplied with adequate phosphorus. They found that fish yields were not significantly correlated with nitrogen input, despite higher phytoplankton biomass. Cool water temperatures apparently inhibited fish growth, rendering the fish unable to take advantage of higher available nutrient supply.

Researchers on the Global Social Sciences Project investigated how and to what extent CRSP research is reaching the institutions that serve farmers, and whether these institutions influence the practices of fish farmers. The study portrayed the institutional context and connections of the CRSP based on information obtained from U.S. scientists, Host Country counterparts, and others knowledgeable about the program. Researchers interviewed over 125 farmers in Rwanda, Honduras, Thailand, and the Philippines, and collected data from cooperating institutions in each Host Country. An economic analysis examined the financial viability of different feeding and pond fertilization approaches associated with several years of parallel experimentation. This study will facilitate the conduct of research that meets farm-level needs in an environmentally and socially sustainable way.

Results from the Global Social Sciences study indicate that tilapia growers in each of the countries face vastly different institutional systems supporting tilapia production. Therefore, the CRSP researchers suggested that CRSP efforts should emphasize infrastructure development and improved functioning of the private sector when the CRSP has the opportunity to do so. Currently, poorly organized markets and distribution systems hinder aquaculture development. As markets for tilapia expand, so will demand for production and support services. The development of private sector marketing services are crucial for sustained aquacultural development. Efforts to enhance the transfer and utilization of CRSP research results will require greater attention to actual and potential pathways of influence and information flow to the farm and village. Although the provision of information directly to end-users is not a mandate of the CRSP, a better understanding of the actual and potential pathways of influence and information flow will help researchers focus their efforts to include appropriate influential institutions as research partners.

DAST researchers continued work on model refinement in the decision support system *POND*© (Version 2.5). These models provide users with the capability of simulating pond aquaculture facilities at three levels of complexity. At Level 1, models are geared toward applied management and rapid analysis of pond facilities. Simulation results agree reasonably well with observed data under a wide range of culture conditions, suggesting that the models used at this level are relatively robust and will likely be useful for a diverse audience, including pond managers, planners, and educators. The water temperature model in *POND*© has been validated by the use of CRSP data from Honduras, Rwanda, and Thailand. The fish bioenergetics model has also been calibrated for *Ictalurus punctatus* (channel catfish), *Colossoma macropomum* (tambaquí), and *Piaractus mitrei* (pacu).

Level 2 models allow for more detailed pond analysis, management optimization, and numerical experimentation. Plankton and nutrient dynamics in ponds are part of this model. Level 3 models explore in greater detail fundamental aspects of pond dynamics such as detailed nutrient transformations in pond water and sediments, as well as atmospheric diffusion processes.

A methodology to enable users to customize *POND*© for alternate culture species and locations has been incorporated directly into the software. Because of the high level of complexity of interactions among variables in the model, manually changing the parameters proved to be extremely time consuming, limiting the use of the software for examining production potential for different pond culture species. An iterative, non-linear, adaptive search method (genetic algorithm or GA) for automatically generating new parameters for the fish growth model has been developed. Adequate convergence to acceptable parameter values was obtained for the three species (channel catfish, tambaquí and pacu) chosen to evaluate GAs as an effective parameter estimation technique.

Another example of the global activities of the CRSP includes the Central Data Base which is used for global analyses and model building. The Data Base is the central repository for data from the CRSP Global Experiments. The Central Data Base has been housed at the University of Hawaii at Hilo since mid-1993. During this reporting period, all incoming data were processed, all data requests were filled, a new data entry manual was drafted, Data Base structure was modified to handle textual data, and the Data Base manager, worked with the Program Management Office on establishing a link to the Data Base on the PD/A CRSP's World Wide Web (www.orst.edu/dept/crsp/homepage.html). During the 1996 Annual Meeting in Thailand the Technical Committee reviewed two proposals to relocate the Central Data Base. The Technical Committee recommended that John Bolte of the OSU DAST team manage the Central Data Base. Beginning April 1996, the Central Data Base will be housed at the Department of Bioresource Engineering at OSU.

The Effects of Fertilization on Growth and Production of Nile Tilapia in Rain-Fed Ponds

Work Plan 7, Thailand Study 1

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Introduction

Semi-intensive production of Nile tilapia *Oreochromis niloticus* commonly utilizes organic and inorganic fertilizers to increase primary production and fish yield. Most experiments on such systems are done at fish culture stations where water supplies are readily available, and water loss through evaporation or by seepage is replaced regularly, often weekly. Rainfall during such experiments may also flush the ponds, reducing nutrient concentrations and improving water quality. In such systems, regular fertilization at high input levels can result in yields approaching 3,500 kg/ha over 5 months, or extrapolated yields of up to 10,000 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Knud-Hansen et al. 1993, Diana 1996). Optimal fertilization rates in Thailand determined to be 28 $kg\ N \cdot ha^{-1} \cdot wk^{-1}$ and an N:P ratio of 4:1 (Knud-Hansen et al., 1993). The fertilization schedule was weekly in these ponds, where combinations of chicken manure and urea or triple super phosphate were used for inputs. Knud-Hansen et al. (1993) believed that inorganic fertilizer produced higher yields than manures at the same loading rates. However, Diana et al. (1994) found reductions in alkalinity in some ponds fertilized with inorganic fertilizers alone which they believed were due to carbon extraction for photosynthesis. No declines in alkalinity were noted in ponds fertilized with manure and inorganic fertilizer or ponds receiving inorganic fertilizer and feed.

The effectiveness of fertilization in rain-fed ponds may differ considerably from ponds receiving water inputs. During the rainy season, these ponds fill and may flush, while during the dry season only evaporation and seepage occur. Rain-fed ponds are generally dug deeper to hold more water during the wet season, and water depth declines during the dry season. Thus, there are two characteristics of importance: increased pond depth and a stagnant water supply. Stagnant water may require less nutrients to maximize fish production, since flushing does not occur. The schedule of fertilization may also differ. However, Knud-Hansen and Batterson (1994) found that fertilization frequencies varying from daily to once every three weeks had no effect on primary production or fish yield in ponds with water inputs.

The purpose of this study was to evaluate fertilization strategies for rain-fed ponds based on strategies developed in ponds with regular water inputs. To accomplish this, four experimental treatments were utilized: (a) fertilization every two weeks with water replacement, (b) fertilization every two weeks without water replacement, (c) fertilization once at the start of culture without water replacement, and (d) fertilization irregularly (when water concentrations of nutrients declined) without water replacement.

Materials and Methods

Data for this study were collected at the Huay Luang Freshwater Fisheries Station located near Udorn (17° 27' N, 102°48' E), approximately 160 km northeast of Bangkok, Thailand. Fifteen ponds used in the experiments were 1600 m^3 in volume, 800 m^2 in surface area, and originally filled to a depth of 2.5 m. All ponds were fertilized with chicken manure (assayed at 89% dry matter, 1.4% N, and 1.2 % P) and enough urea and triple super phosphate (TSP) to produce an N:P ratio of 4:1 and total N addition to the desired treatment level.

Table 1. The biomass (g), number, and mean weight (g) of tilapia stocked and harvested from each pond.

Pond	At Stocking			At Harvest		
	Mean Weight	Number	Biomass	Mean Weight	Number	Biomass
A1	18.8	1600	30	194.5	1508	308
A2	17.5	1600	28	178.2	1591	299
A3	15.1	1600	24	263.0	1301	333
B1	17.3	1600	28	220.5	1461	347
B2	19.0	1600	30	218.4	1599	368
B3	13.3	1600	21	232.8	1480	346
C1	12.2	1600	20	47.7	1194	55
C2	14.2	1600	23	30.0	1281	36
C3	14.1	1600	23	23.5	659	14
D1	13.3	1600	21	158.5	1467	240
D2	14.6	1600	23	153.5	1540	235
D3	15.6	1600	25	151.3	1068	173

Four fertilization treatments were used: a) weekly fertilization with water addition, b) weekly fertilization without water addition, c) one fertilization at the start without water addition, and d) fertilization at irregular intervals dependent on the nutrient concentrations of the pond water, without water addition. Treatments A, B, and D were fertilized with 22.5 kg chicken manure, 4.5 kg urea, and 1.4 kg TSP per pond at each dosing. These rates equaled 280 $kg \cdot ha^{-1} \cdot wk^{-1}$ manure, 56 $kg \cdot ha^{-1} \cdot wk^{-1}$ urea, and 17.5 $kg \cdot ha^{-1} \cdot wk^{-1}$ TSP. Treatment C received 89 kg chicken manure, and 2.4 kg urea at the start of the experiment, with no further additions. Treatment D received a new dose of fertilizer when dissolved inorganic nitrogen (DIN) levels in the water declined below 0.5 mg/L.

Each fertilization treatment was done in triplicate. Sex-reversed Nile tilapia were stocked on 8 September 1994. Stocking density was 2 fish/m^2 (1600 fish per pond) and size at stocking averaged 15 g (Table 1). Every two weeks, 20 fish from each pond were sampled, individually weighed (to 1 g), and measured in length (to 1 mm). Growth rate was calculated as the increase in weight per day between sampling periods.

Physical and chemical data were collected in a similar manner to earlier experiments (Diana et al. 1991b, 1994). Meteorological data, including solar radiation, rainfall, and wind speed were collected daily. For most water analyses, combined samples encompassing the entire water column were taken from walkways extending to the center of the ponds. Pond water analyses, including temperature, dissolved oxygen (both taken at the top, middle, and bottom of the water column), ammonia, nitrate/nitrite, soluble-reactive phosphorus, total phosphorus, alkalinity, pH, Secchi-disk depth, and chlorophyll-*a* content were conducted biweekly using standard methods (see APHA 1980 and Egna et al. 1987 for detailed descriptions of methods). Vertical distribution of dissolved oxygen, temperature, pH, alkalinity, and ammonia was determined at 0530 hr, 1200 hr, 1800 hr, 2400 hr, and 0600 hr in each pond. These diel analyses were repeated about monthly on water from depths of 5 cm (top), 30 cm, 100 cm, 150 cm, and 180 cm (bottom). Maximum temperature and oxygen differentials were calculated as the difference between top and bottom measurements at 1800 hr.

Table 2. Growth (g/d), survival (%), yield ($kg \cdot ha^{-1}$), and forecasted annual yield ($kg \cdot ha^{-1} \cdot yr^{-1}$) for tilapia from each pond.

Pond	Growth	Survival	Yield	Annual Yield
A1	0.75	94	3479.38	5427.23
A2	0.69	99	3386.90	5282.99
A3	1.06	81	3853.85	6011.35
B1	0.87	91	3988.13	6220.79
B2	0.85	100	4215.90	6576.08
B3	0.94	93	4059.15	6331.58
C1	0.15	75	446.08	695.80
C2	0.07	80	168.50	262.83
C3	0.04	41	-110.79	-172.82
D1	0.62	92	2734.13	4264.77
D2	0.59	96	2642.83	4122.36
D3	0.58	67	1851.30	2887.71

Primary production was determined by oxygen changes in the ponds, using methods described by Piedrahita (1988). Daily oxygen production was corrected for diffusion and nocturnal respiration. The overall oxygen production (Gross Primary Production) was then converted to carbon synthesis by relative molecular weights.

One interest in this study was to determine the accumulation of nutrients and metabolites in pond waters. This accumulation was estimated by averaging values over the first two water sampling periods (8 and 22 September 1994) as estimates of initial water chemistry, then over the last two sampling periods (9 and 23 March 1995) as the final water chemistry.

Statistical analyses were conducted using SYSTAT (Wilkinson 1990). Overall growth (g/day) and net yield (kg) were calculated for each pond. Average overall values for physical and chemical parameters and total fertilizer input were also calculated. Multiple regressions between growth rate and design variables (fertilizer input, depth) were done to test main effects. Because many of the chemical variables were interrelated, residuals of the above regression were correlated to each physical or chemical variable. Significantly correlated variables were then examined for auto correlation, and acceptable variables input to the multiple regression to evaluate determinants of fish growth. Variables were included in the regression if $p < 0.10$. Treatment effects on fish or chemical variables were tested with the biweekly data set by ANOVA and Tukey's multiple range test. Accumulation or loss of materials in the water over time was estimated by comparing initial and final values with a t-test, using data for each treatment. All differences were considered significant at an alpha of 0.05.

Results

There were significant differences in growth rate among treatments, with treatments A and B having the highest growth rate, D with an intermediate growth, and C with no growth at all (Figure 1, Table 2). Growth rates in treatments A and B averaged 0.86 g/d. Survival was variable among ponds, but was not significantly different among treatments and averaged 84%. Yield showed similar trends to growth rate, with the highest yields occurring in Treatments A and B, the lowest in C, and statistically significant differences.

Table 3. Treatment-related values for physical and chemical variables measured throughout the experiment. Values with the same superscript are not significantly different. Variable names with superscripts had no significant differences among treatments.

Variables	A	B	C	D
Alkalinity^{1}	93.4	92.5	96.8	89.4
Chlorophyll *a*	75.8^{1}	59.3^{1}	12.8	59.2^{1}
Depth	248.9	207.8^{1}	194.4^{1}	205.7^{1}
Ammonia	0.345^{1}	0.405^{1}	0.047^{1}	0.314^{1}
Nitrite	0.374^{1}	0.410^{1}	0.059^{2}	0.107^{2}
Nitrate	0.438^{1}	0.461^{1}	0.071^{2}	0.189^{2}
Soluble reactive P	0.448^{1}	0.407^{1}	0.141^{2}	0.217^{2}
Secchi disk depth^{1}	34.7	34.4	34.6	34.4
DIN	1.16^{1}	1.28^{1}	0.177^{2}	0.611^{3}
Total P	0.533^{1}	0.477^{1}	0.113^{2}	0.295^{3}
Total suspended solids^{1}	34.4	40.5	28.8	38.3
Total volatile solids	12.8^{1}	11.7^{1}	5.8	11.9^{1}

Depth of water declined throughout the experiments in ponds without water replacement (Figure 2). There was no effect of fertilization on water depth. Depth averaged 242 cm upon initiation of experiments and declined to 158 cm at harvest in ponds without water replacement.

Several physical and chemical variables also varied by treatment. Nitrogen and phosphorus levels in water differed by treatment, with treatments A and B showing similar levels, which were often significantly higher than D, which in turn was often significantly higher than C (Table 3). This was true for ammonia, nitrite, nitrate, DIN, soluble-reactive phosphorus, and total phosphorus. Also, chlorophyll- *a* content differed significantly by treatment in the same manner.

Fish growth rates were strongly correlated to the treatment variable of manure input but not to water depth (Table 4). This correlation was very strong ($R^2 = 0.89$), and residuals were not correlated to any physical or chemical variables. Survival was not significantly correlated to the design variables. Yield was again strongly and significantly correlated to fertilizer input only ($R^2 = 0.94$), and the residuals were not correlated to any physical or chemical variables.

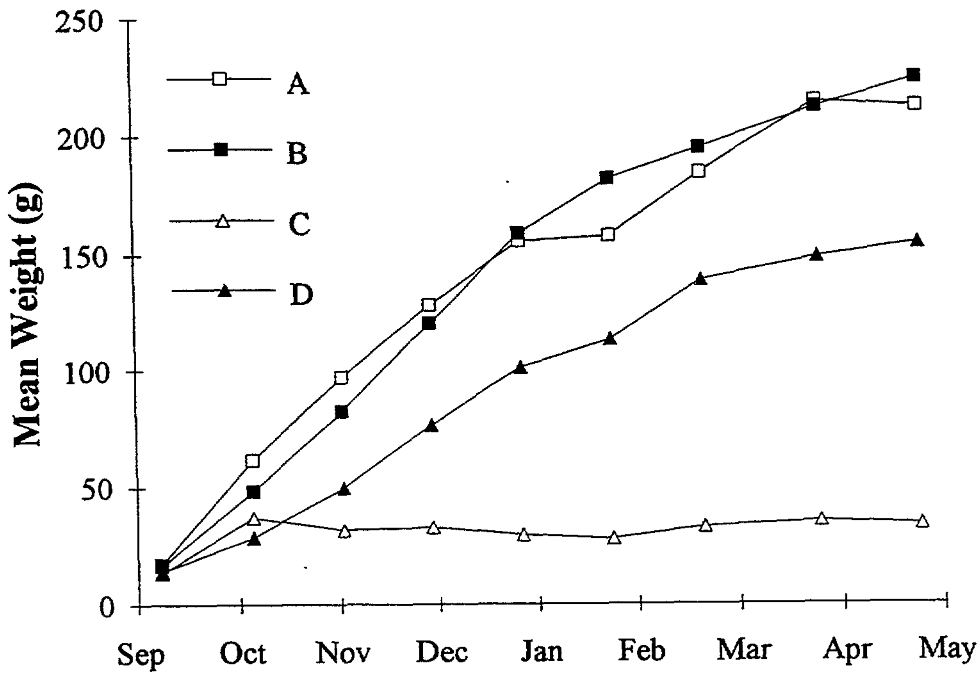

Figure 1. Changes in mean weight throughout the experiment for fish from each fertilization treatment.

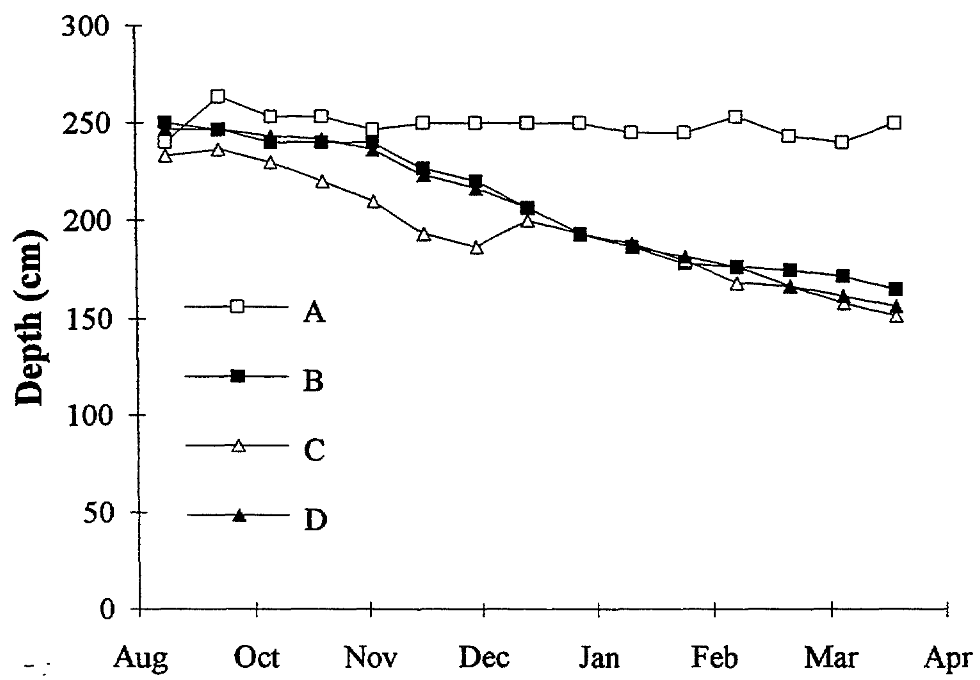

Figure 2. Changes in mean water depth throughout the experiment for ponds from each treatment.

Table 4. Results of multiple regression analyses for fish growth (g/d), survival (%), and yield (kg/pond).

Variable	Coefficient	P
	Growth rate - R^2 = 0.890, p = 0.001	
Constant	0.292	0.487
Manure input	0.001	0.001
Depth	-0.002	0.494
	Survival - R^2 = 0.337, p = 0.138	
Constant	0.692	0.191
Manure input	0.000	0.063
Depth	-0.000	0.905
	Yield - R^2 = 0.940, p = 0.001	
Constant	130.067	0.273
Manure input	0.562	0.001
Depth	-0.801	0.204

One concern in rain-fed ponds was that nutrients and metabolites might accumulate at deleterious levels as time progressed. Treatments A and B showed significant accumulation of nutrients over time, with final values for nitrite, nitrate, DIN, soluble-reactive phosphorus, and total phosphorus being much higher than initial values (Table 5). However, ammonia levels (a metabolite) did not increase significantly, nor did chlorophyll- *a* content. Treatment C showed significant declines in alkalinity, ammonia, nitrate, soluble-reactive phosphorus, total suspended solids, and total volatile solids over time. Treatment D had significant accumulations of nitrite, soluble-reactive phosphorus, and total phosphorus. Accumulations of total phosphorus and DIN were sporadic over time, especially in treatment D with irregular fertilization, but increased more or less continuously in treatments A and B (Figure 3).

Discussion

Fertilization strategies for shallower ponds with water addition produced the best growth and yield of fish in this experiment. Fish production was similar at high nutrient inputs, regardless of water addition. Attempts to add nutrients as supplies dwindled were not as successful in producing fish. These results all indicate that fertilization guidelines may be much more generally applicable than to ponds with similar depths and water management systems.

Control of water depth by addition had no effect on fish production or accumulation of materials in pond waters. Apparently, the nutrient conditions are so dependent on organisms in the pond that evaporation has no effect in concentrating nutrients. Szyper et al. (1991) found that depths of 0.6, 1.0, and 1.5 m had no effect on fish yield (per unit area, not volume). When deeper ponds received nutrient inputs and stocking densities on a per volume basis, they produced higher yields per unit area. These experiments support the results of the present study, although depth was controlled by water addition in the Szyper et al. (1991) study. Also, they used shallower ponds than the present study, although the final depths in treatments B, C, and D averaged 1.5 m.

Fertilization guidelines for shallower (1m deep) ponds are similar when expressed on a per area or per volume basis. However, as pond depth increases, fertilization per unit water volume declines if guidelines are developed per unit area. The present study used guidelines produced on an areal basis. Growth rates (0.84) and annual yields (5974 $kg \cdot ha^{-1} \cdot yr^{-1}$) were somewhat lower than found in

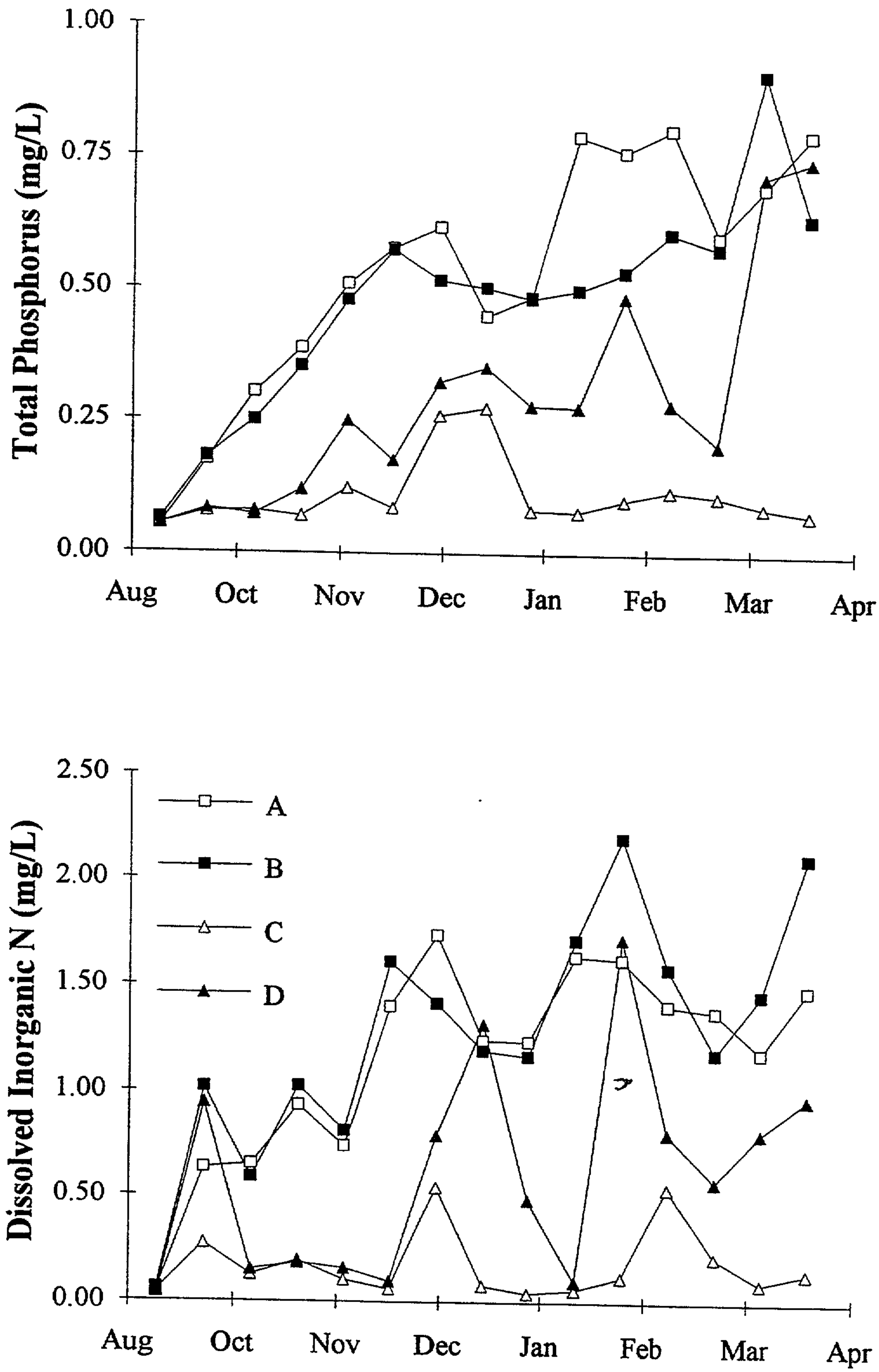

Figure 3. Changes in total phosphorus (top) and dissolved inorganic nitrogen (bottom) throughout the experiment in ponds from each treatment.

Table 5. Time-related values for physical and chemical variables measured throughout the experiment. Treatments with two values indicate significant time differences, and are listed with beginning values first; ns = no significant differences between initial and final values.

Variables	A	B	C	D
Alkalinity	ns	ns	77.3 102.0	ns
Chlorophyll *a*	ns	ns	ns	ns
Depth	ns	248.3 168.3	235 155	ns
Ammonia	ns	ns	0.122 0.005	ns
Nitrite	0.032 0.558	0.055 0.523	ns	0.028 0.095
Nitrate	0.065 0.592	0.115 0.735	0.023 0.090	ns
Soluble reactive P	0.030 0.527	0.115 0.735	0.017 0.040	ns
Secchi disk depth	ns	ns	ns	0.027 0.180
Dissolved inorganic N	0.343 1.333	0.540 1.782	ns	ns
Total P	0.114 0.743	0.123 0.770	ns	0.068 0.728
Total suspended solids	ns	ns	30.7 15.3	ns
Total volatile solids	ns	ns	11.0 5.4	ns

other experiments (Diana et al. 1991a, 1991b, Szyper et al. 1991, Green 1992). Possibly, more nutrient inputs could be utilized to account for the larger volume of water in these ponds and further increase productivity. The regression analysis indicated both growth and yield increased significantly with manure input. Using that regression, a manure input of 316 $kg \cdot ha^{-1} \cdot wk^{-1}$ would result in fish growth of 1 g/d and yield of 7039 $kg \cdot ha^{-1} \cdot yr^{-1}$ which is similar to the previous results in shallower ponds.

Utilizing water nutrient levels as an indicator of when to fertilize resulted in lower fish production. Since nutrient levels were only measured biweekly, the only adjustment which could be made was to reduce nutrient input. More frequent measures of nutrients might allow such a system to more efficiently utilize nutrients, although it would require much more labor.

Ponds which were fertilized only once showed no growth. In these ponds, nutrients were quickly utilized then primary production and growth declined dramatically. Growth rate (0.086 g/d) and annual yields (261 kg · ha^{-1} · yr^{-1}) were considerably lower than earlier results in low nutrient input ponds (Diana et al. 1991a, 1991b), and are also well below expectations for poorly fertilized ponds (Diana 1996).

Contrary to expectations, stagnant ponds with no water replacement do not accumulate nutrients and metabolites at unreasonable rates. Fertilization guidelines could be improved by accounting for differences in depth, but otherwise guidelines from ponds receiving water inputs seem appropriate for production in rain-fed ponds. Szyper et al. (1991) also found that fertilization guidelines accounting for volume of water in ponds could increase fish production in ponds of varying depths.

Acknowledgments

This project was conducted with cooperation of the National Inland Fisheries Institute, Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. Wattana Leelapatera aided in logistics and data evaluation. The study was conducted at the Huay Luang Freshwater Fisheries Station. Data collection and lab analyses were done by V. Tansakul, S. Auworatham, W. Muthuwana, and assistants. B. Diana aided in data analysis and text preparation. This research is a component of the Pond Dynamics/Aquaculture CRSP supported by the Agency for International Development Grant No. DAN-4023-G-SS-2074-00, and by contributions from participating institutions.

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Nitrogen Fertilization in the Presence of Adequate Phosphorus

Workplan 7, Honduras Study 4D

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Introduction

Primary production and fish production in organically fertilized ponds were increased significantly when supplemented with sufficient inorganic nitrogen to raise total weekly nitrogen inputs to 28 kg/ha (Teichert-Coddington and Green, 1993; Teichert-Coddington et al., 1993). Nitrogen was apparently limiting productivity, but optimum nitrogen fertilization rates were not determined. The objective of this study was to quantify limiting levels of nitrogen in semi-intensive tilapia culture ponds supplied with adequate levels of phosphorus.

Materials and Methods

A completely randomized design was used to test 4 levels of inorganic nitrogen fertilization. Earthen ponds, 0.1 ha and 0.9 m mean depth, were fertilized with urea to supply 0, 7, 14, or 28 kg/ha-wk nitrogen. Each nitrogen level was replicated three times. All ponds were also fertilized with 8 kg/ha-wk of phosphorus as triplesuper phosphate. Total weekly fertilizer input was divided into two doses. Fertilizer was first dissolved in water before being applied to the pond.

Ponds were stocked with 11-g *Oreochromis niloticus* fingerlings at 2 fish/m^2, and guapote tigre at 500/ha to control reproduction. Fish were sampled monthly for growth.

Water sub-samples were collected from various locations in the pond with a column sampler and combined. The composite sample was analyzed according to standard methods (American Public Health Association (APHA) et al., 1992), unless otherwise indicated. Chlorophyll-*a* (Boyd and Tucker, 1992), filterable phosphorus, ammonia nitrogen, DO, and pH were determined weekly. Total phosphorus and total nitrogen by combined alkaline oxidation (Grasshoff et al., 1983), total alkalinity (titration to pH 4.5), total hardness, and primary productivity (free-water method) were determined monthly.

Ponds were stocked on 28 July 1994 and harvested after 146 d on 21 December 1994. Data were analyzed by ANOVA (Gagnon et al., 1989). Linearity of response to nitrogen fertilization was investigated by linear contrasts within the ANOVA model. Dunnett's T single tail test was used to compare means at different levels of nitrogen fertilization with the control. Differences were declared significant at alpha = 0.05.

Results

Nitrogen fertilization resulted in linear increases of total ammonia and total nitrogen (Figure 1, Table 1), as might be expected. Mean filterable phosphate was not significantly influenced by nitrogen fertilization (Figure 2), although the control mean concentration tended to be higher than the nitrogen fertilized treatments. The arithmetic difference between the control and nitrogen fertilized treatments became particularly large during the last month of growth (Figure 2). Total phosphorus did not significantly change with nitrogen fertilization (Figure 2). Total hardness and total alkalinity did not respond linearly to nitrogen fertilization (Figure 3). However, total hardness of each nitrogen treatment was significantly lower than the control. Only the

Table 1. Mean water quality variables for earthen ponds fertilized with 8 kg/ha-wk phosphorus as triple-super phosphate, and urea to supply 0, 7, 14, or 28 kg/ha-wk nitrogen.

Treatment	Total ammonia (mg/l)	Total nitrogen (mg/l)	Filterable phosphate (mg/l)	Total P (mg/l)	Total alkalinity (mg $CaCO_3$/l)	Total hardness (mg $CaCO_3$/l)	Chlorophyll *a* (µg/l)	Secchi disk visibility (cm)	Dissolved oxygen (mg/l)
0	0.051	2.29	1.911	1.97	79.7	57.4	100.3	26.1	4.7
7	0.099	3.14	1.170	1.99	65.5	43.4	195.2	19.6	4.1
14	0.180	4.02	1.144	1.84	66.3	37.9	291.5	18.4	4.3
28	0.303	5.97	0.921	1.95	69.2	41.1	393.1	16.5	4.0
Contrasts									
Linear	hs	hs				s	hs		
Quadratic								s	

s = significant ($P < 0.05$); hs = highly significant ($P < 0.01$)

lowest nitrogen treatment resulted in significantly lower total alkalinities than the control. Mean early morning dissolved oxygen concentrations were not significantly different among treatments.

Indicators of primary productivity responded positively to nitrogen fertilization. Chlorophyll *a* increased linearly with fertilization, and Secchi disk visibility decreased curvilinearly (Figure 4).

Fish growth did not respond significantly to nitrogen fertilization (Table 2, Figure 5). There was a tendency for both mean fish weight and production to increase at the 14 kg/ha treatment, but decrease thereafter. Fish growth was significantly curvilinear in all treatments (Figure 5).

Discussion

Primary productivity was clearly promoted by nitrogen fertilization in the presence of adequate phosphate. Phosphorus was high in all treatments with mean dissolved inorganic phosphate ranging between 0.92 and 1.91 mg/l for all treatments. The control treatment reached mean concentrations higher than 2.5 mg/l. Nitrogen fertilized treatments tended to have lower phosphate concentrations because of absorption by a higher biomass of phytoplankton. After the first month of production when phytoplankton had adequate time to become established, filterable phosphate significantly decreased with increased chlorophyll *a* ($P < 0.0001$; $R = 0.45$). A clear point was not established for which

Table 2. Mean tilapia production for earthen ponds fertilized with 8 kg/ha-wk phosphorus as triplesuper phosphate, and urea to supply 0, 7, 14, or 28.

Treatment	Mean fish weight (g)	Total production (kg/ha)	Survival (%)
0	78.6	1412	86.7
7	70	1345	92.3
14	98.3	1664	82.5
28	64.4	1114	85.1

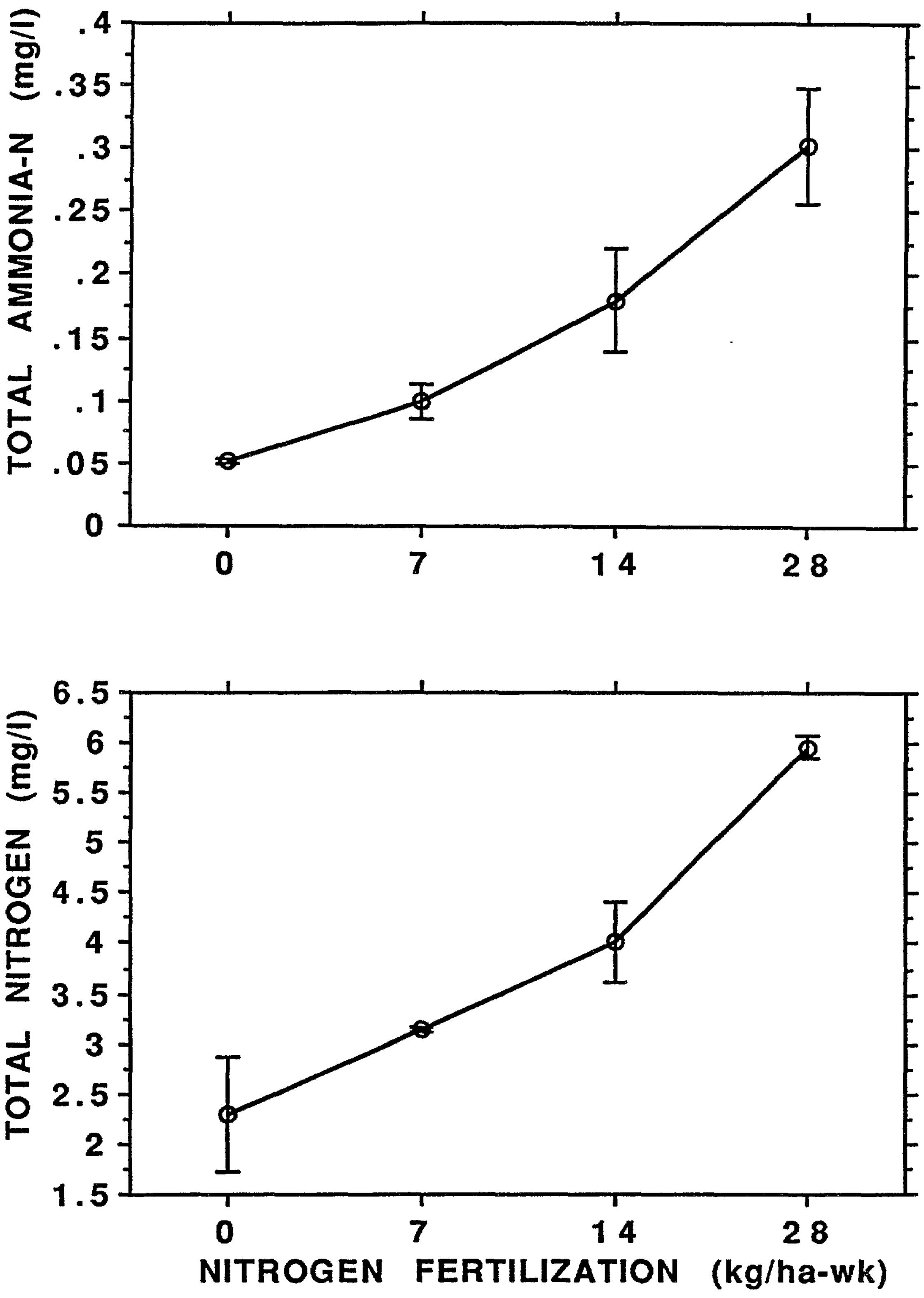


Figure 1. Mean total ammonia nitrogen and total nitrogen for each level of nitrogen fertilization in tilapia ponds supplied with 8 kg P/ha/wk. Bars around the mean indicate standard error.

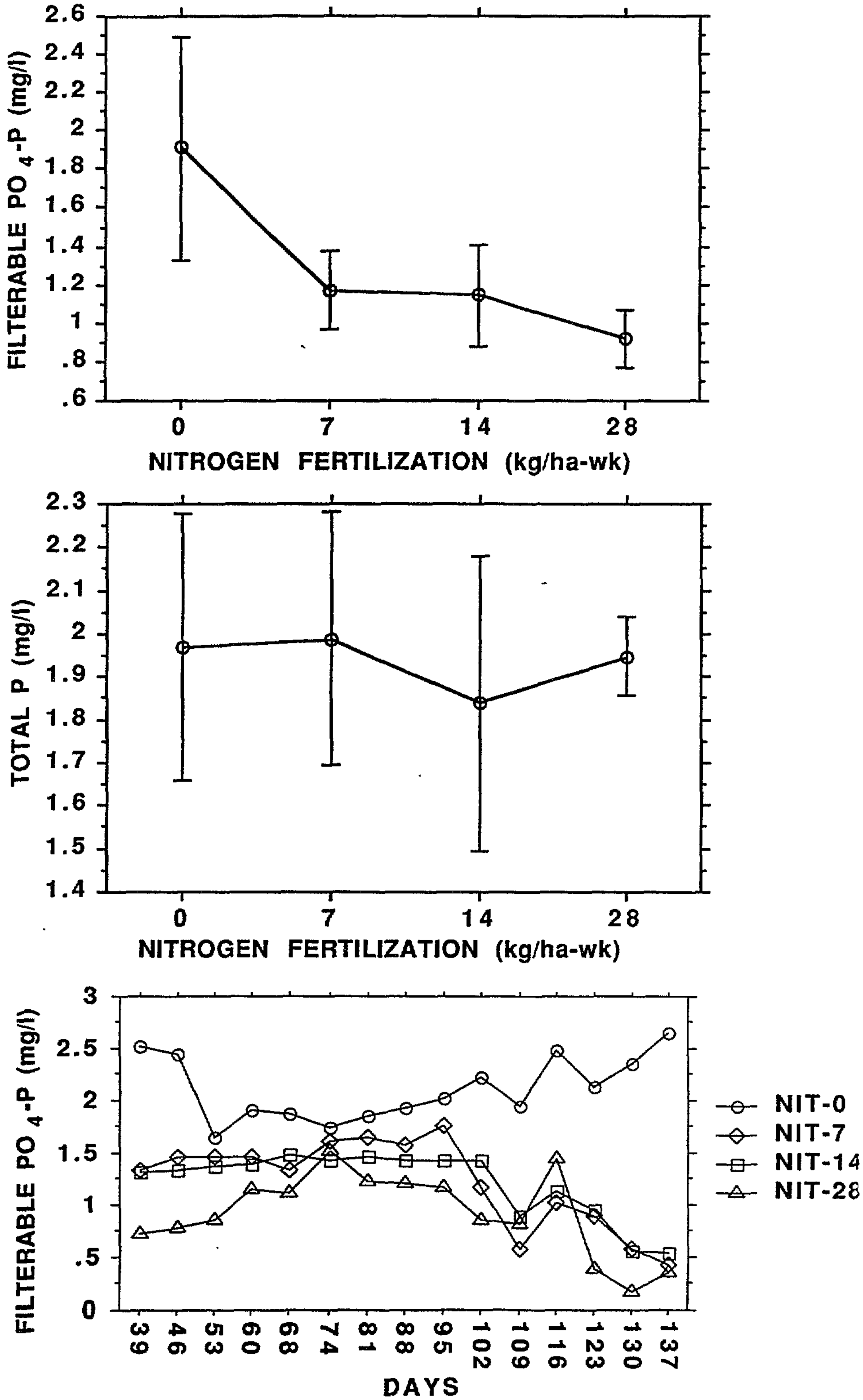


Figure 2. Mean filterable phosphate over time, and mean total phosphorus and filterable phosphate for each level of nitrogen fertilization in tilapia ponds supplied with 8 kg P/ha/wk. Bars around the mean indicate standard error.

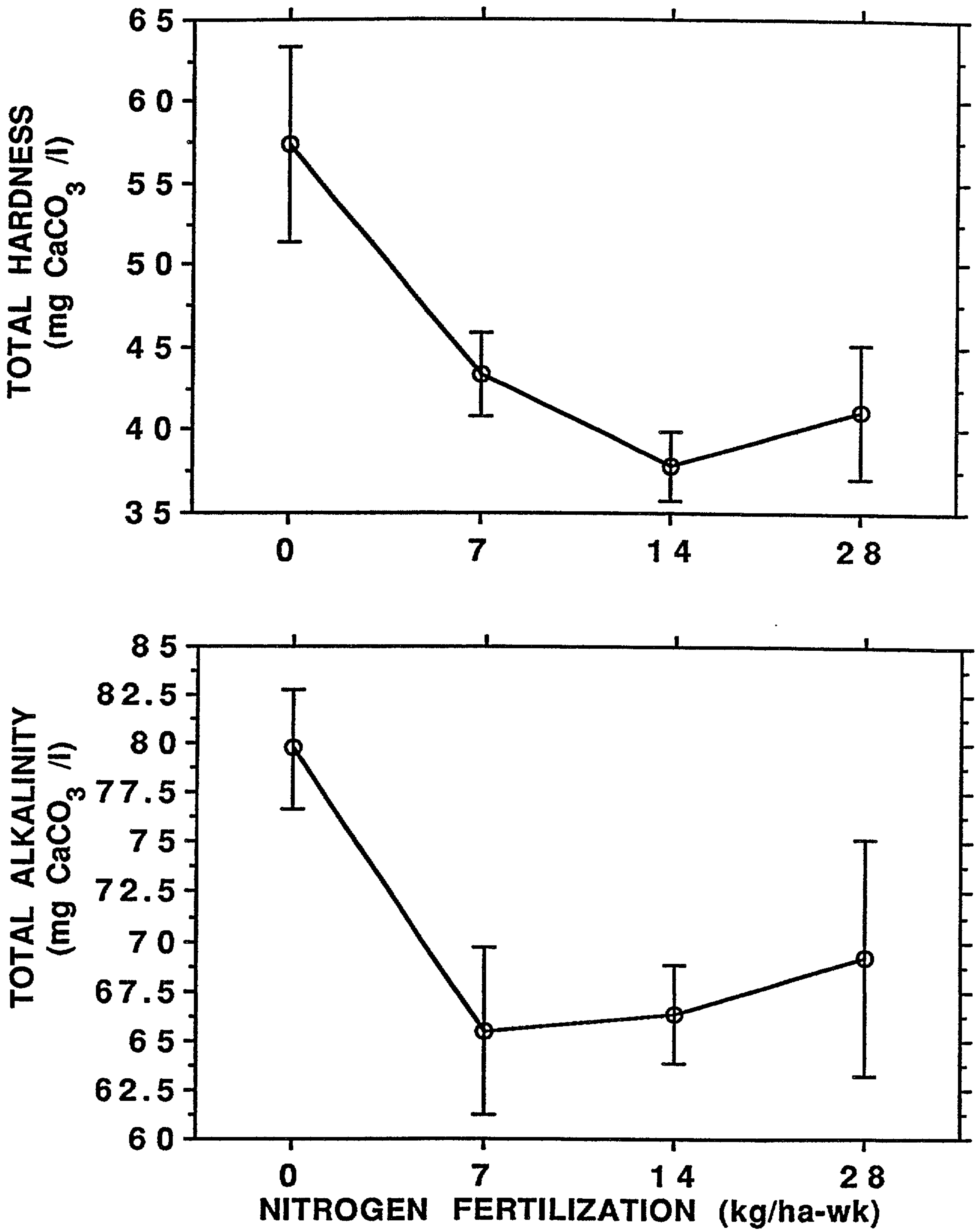


Figure 3. Mean total hardness and total alkalinity for each level of nitrogen fertilization in tilapia ponds supplied with 8 kg P/ha/wk. Bars around the mean indicate standard error.

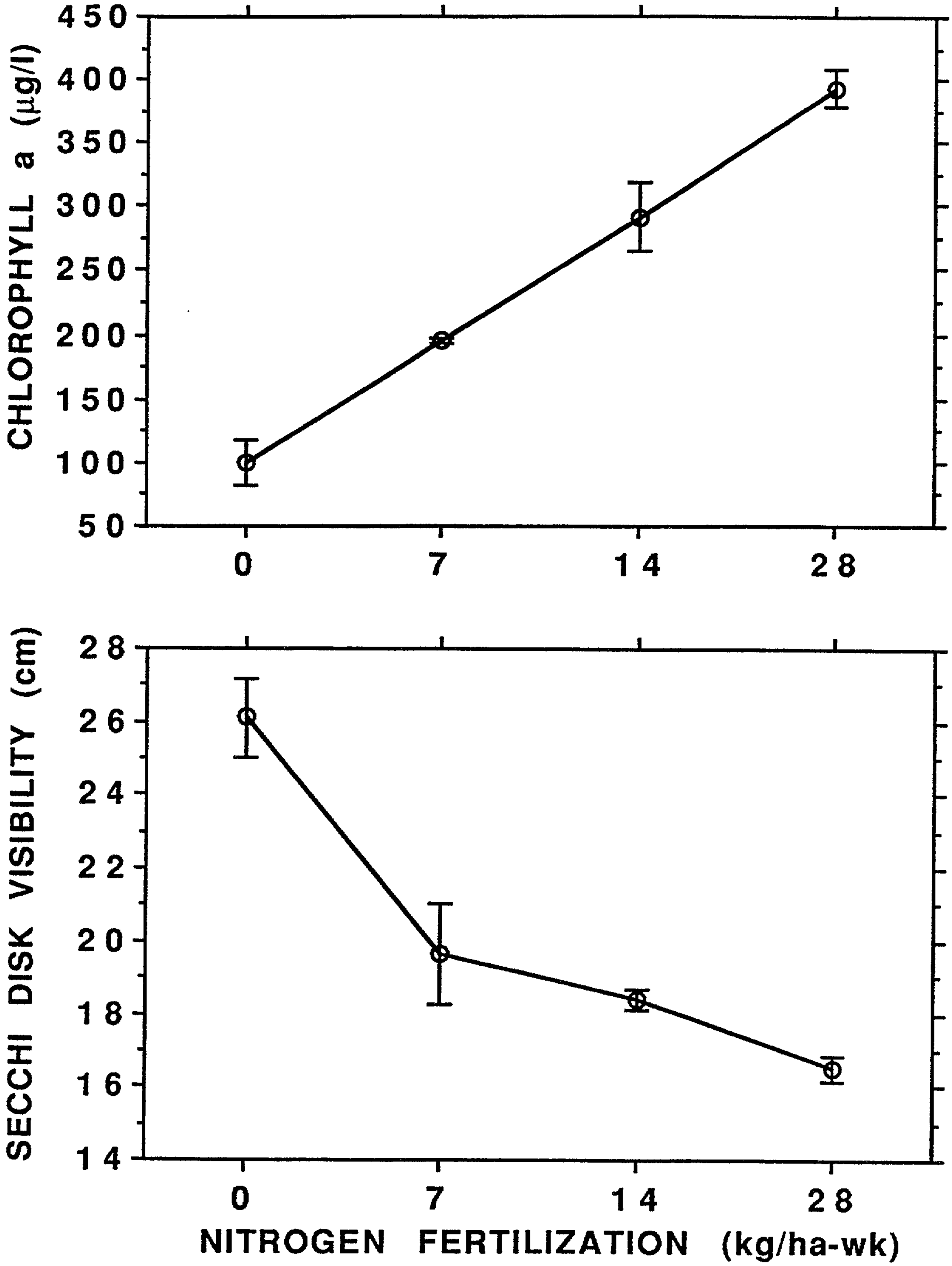
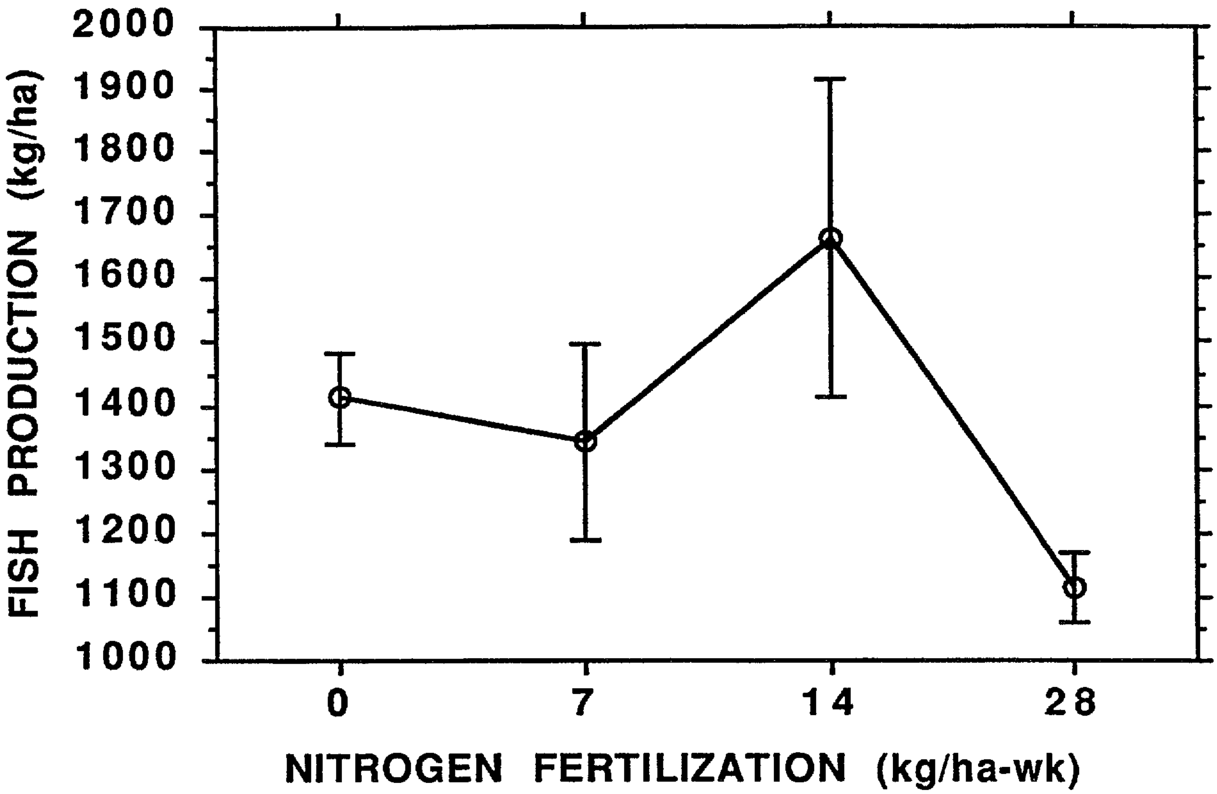


Figure 4. Mean chlorophyll *a* and Secchi disk visibility for each level of nitrogen fertilization in tilapia ponds supplied with 8 kg P/ha/wk. Bars around the mean indicate standard error.



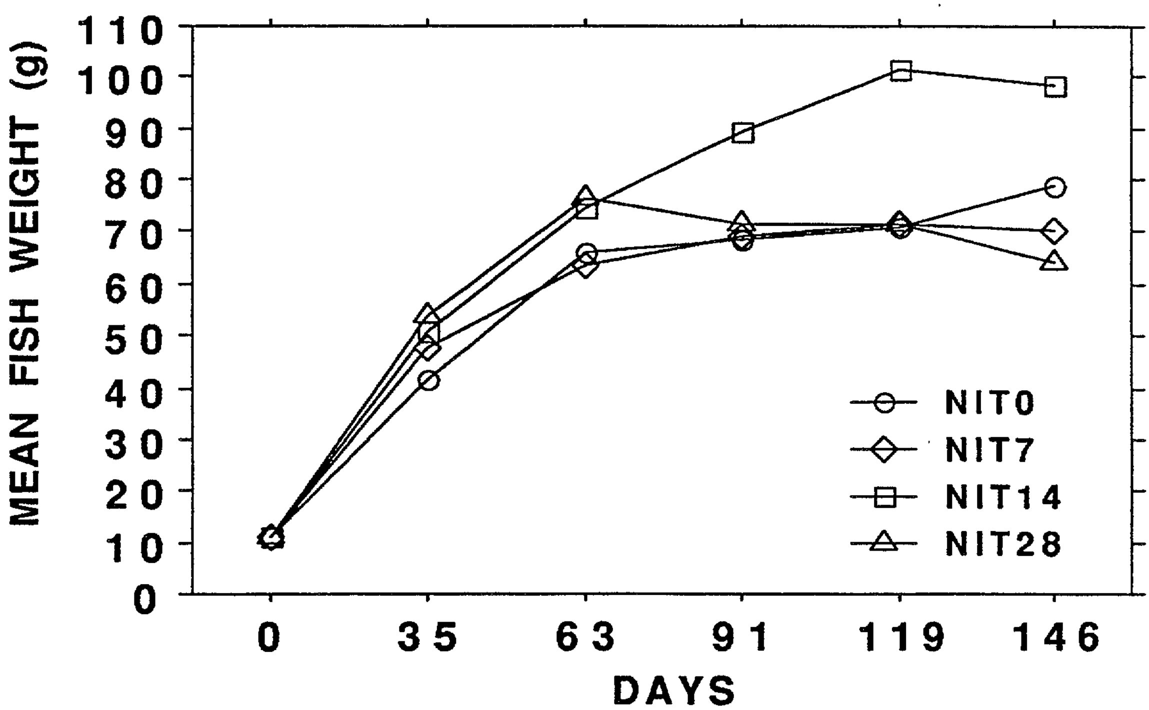


Figure 5. Mean tilapia growth over time, and mean total fish production for each level of nitrogen fertilization in tilapia ponds supplied with 8 kg P/ha/wk. Bars around the mean indicate standard error.

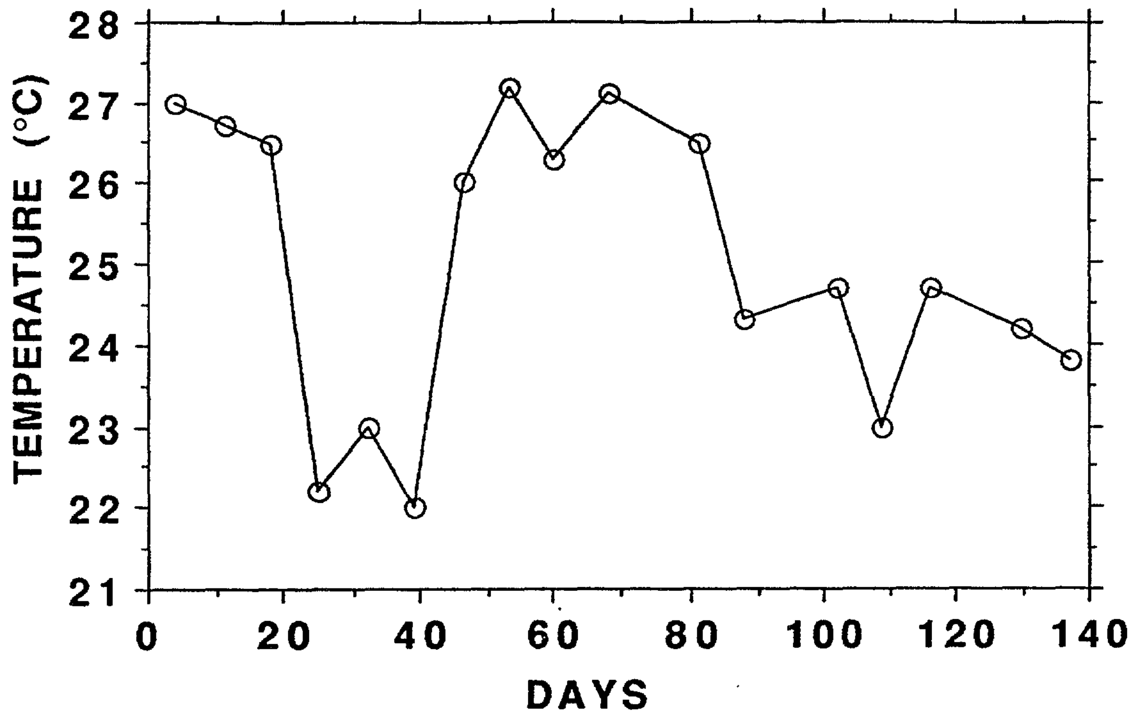


Figure 6. Mean early morning pond water temperature during the experiment.

nitrogen no longer limited primary productivity. However, occasionally high total ammonia levels (> 0.9 mg/l) in the highest nitrogen treatment indicated that ponds were approaching nitrogen saturation.

Despite an increase in primary productivity with nitrogen fertilization, a significant increase was not seen in tilapia production. It is suspected that cool water temperatures during the trial (Figure 6) inhibited fish growth. Growth leveled out after 90 d, about when water temperatures also dropped for a prolonged period. Fish were unable to take advantage of higher available nutrient supply. Indeed, fish production was low for all treatments. In 1992, a similar combination of inorganic P and N during the cool season yielded 1850 kg/ha (Teichert-Coddington et al., 1993), compared with 1664 kg/ha for the highest treatment yield during the current study. In 1991 (Teichert-Coddington et al., 1992), nitrogen supplementation of ponds fertilized with chicken litter significantly increased primary productivity, but did not affect tilapia yield of tilapia stocked at $1/m^2$, after 126 d of growth. It was concluded that fish were stocked at too low a rate and grown for too short a period to take advantage of the extra food supply. A subsequent experiment supported these conclusions. Ponds were stocked with tilapia at $2/m^2$, and fertilized with chicken litter and various levels of inorganic nitrogen (Teichert-Coddington and Green, 1993). Supplemental nitrogen inputs resulted in significant increases in primary production, and fish production was significantly increased at the C6:N1 input ratio resulting in record yields of 3500 kg/ha in 150 d.

Acknowledgments

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Experimental Evaluation of Lime Requirement Estimators for Global Sites

Work Plan 7, Africa Study B

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(Printed as Submitted)

Introduction

Aquaculture ponds with acid bottom muds and soft waters are commonly treated with lime to raise soil pH and base saturation levels and to increase the alkalinity of the pond water to an acceptable level. Pond mud pH readings of less than about 6.0 or pond water alkalinities of 20 mg $CaCO_3$/L or less are usually taken as indications that a given pond needs to be limed (Boyd 1979). Aquaculturists have used a number of methods (both agricultural and aquacultural) to estimate the amount of lime that should be added to ponds. Agricultural methods generally estimate the lime requirement (LR) for raising soil pH to a particular level, whereas aquacultural methods go a step further by estimating the LR for raising pond water alkalinity to a desired level.

Boyd (1974) showed that particular relationships exist between soil pH and soil base saturation and between soil base saturation and water hardness in fish pond muds in Alabama, and that these relationships can be used to estimate the LRs of ponds in that region. Boyd warned, however, that the relationships found for Alabama ponds may not hold for ponds in other areas. Work on agricultural soils by a number of workers, notably Mehlich (1942, 1943), has suggested that the relationship between soil pH and soil base saturation may in fact be dependent on the type of soil, that is on the amount of clay and organic matter present and on the mineralogy of the clay fraction. The work of Bowman and Lannan (1995) supports the hypothesis that this relationship does indeed vary with soil type. If different soil types have different relationships between soil pH and soil base saturation, then a logical question is whether different methods for estimating pond lime requirements should be used for different soil types. This study was designed to evaluate the suitability of several different LR estimators for a wide variety of soils.

Materials and Methods

Soil samples from aquaculture ponds or other wetland areas in Thailand, Kenya, and Rwanda were collected, characterized, and treated in laboratory microcosms to evaluate the suitability of several LR estimators for them. Prior to treatment each soil sample was air-dried and crushed to pass a 2mm sieve. Baseline analyses were done to determine pH, particle size distribution, exchangeable bases (calcium, magnesium, potassium, and sodium), CEC, and acidity. The LR of each soil was estimated by the following methods: SMP-1 (single-buffer method of Shoemaker et al., 1962), SMP-2 (double-buffer modification of SMP method described by McLean, 1982), SMP kit ("lime requirement module" from HACH), Boyd (1979), Pillay and Boyd (1985), and POND (Bolte et al., 1994).

The experiments were conducted in a constant temperature room at the Oak Creek Laboratory of Biology, Oregon State University, Corvallis, Oregon. Glass beakers with a capacity of 800 mL were used to test the LR estimates obtained using the methods noted above. Each beaker was filled with 750 mL of soft water (alkalinity of approximately 10 mg $CaCO_3$/L). The appropriate amount of agricultural limestone was thoroughly mixed with twenty-five grams of soil and then added to the solution. The soil-lime-water mixture was stirred vigorously with a glass rod for ten seconds to begin the experiment. An unlimed treatment was also prepared for each soil. For some soils an additional, high lime treatment was included. Each treatment was applied in triplicate. Water temperatures in the beakers were maintained at between 23 and 26°C. Samples of approximately 12.5 mL were removed after 1, 3, 7, 14, 21, and 28 days for determination of total alkalinity. Alkalinity was determined according to the methods described in Standard Methods (APHA et al., 1989). Three runs of the experiment were conducted between November 1994 and July 1995.

FOOTNOTE: 1 The lime requirement module is part of the HACH Soil and Irrigation Water Kit, Model SIW-1, Cat. No. 24960-00. HACH Company, Loveland, Colorado.

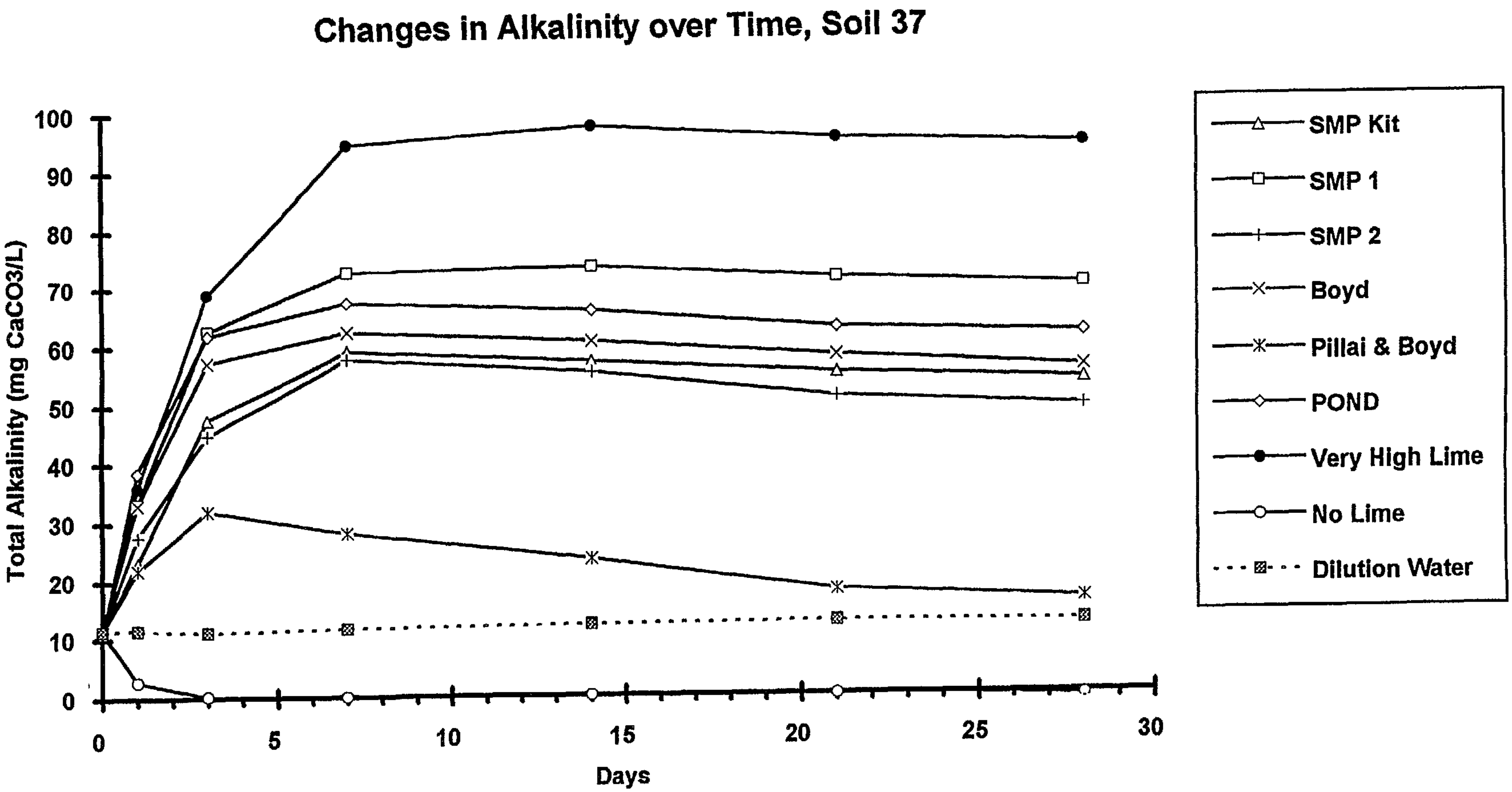

Figure 1. Changes in alkalinity over time for microcosms containing Soil 37. All lime requirement estimators provided adequate initial levels of alkalinity in the microcosms, but alkalinity in the Pillai and Boyd treatment fell to unsatisfactory levels by about 15 days after treatment.

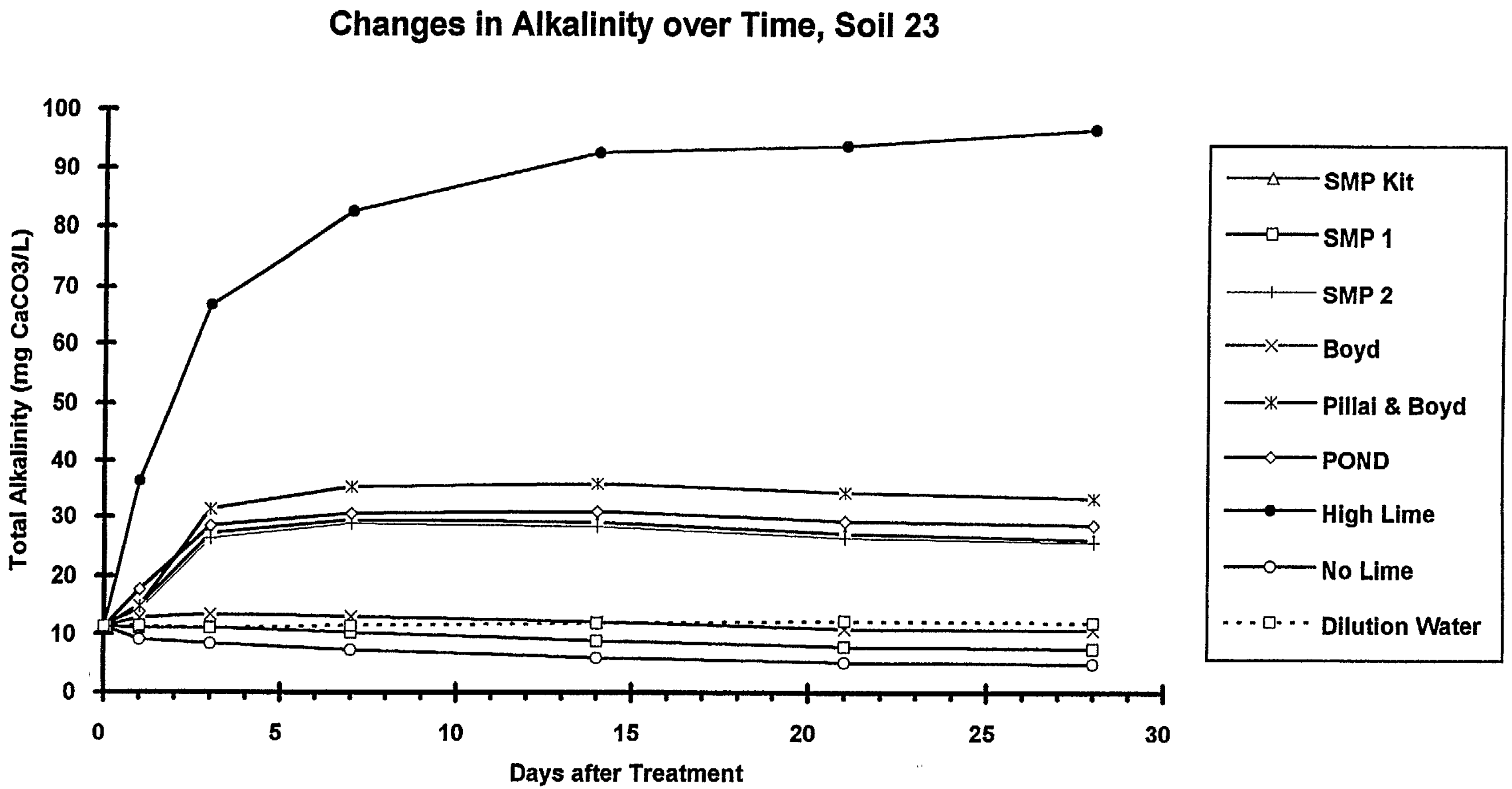

Figure 2. Changes in alkalinity over time for microcosms containing Soil 23. All lime requirement estimators except SMP-1 and Boyd provided adequate levels of alkalinity in this soil throughout the experimental period. In microcosms where no lime was applied alkalinity decreased steadily throughout the course of the experiment.

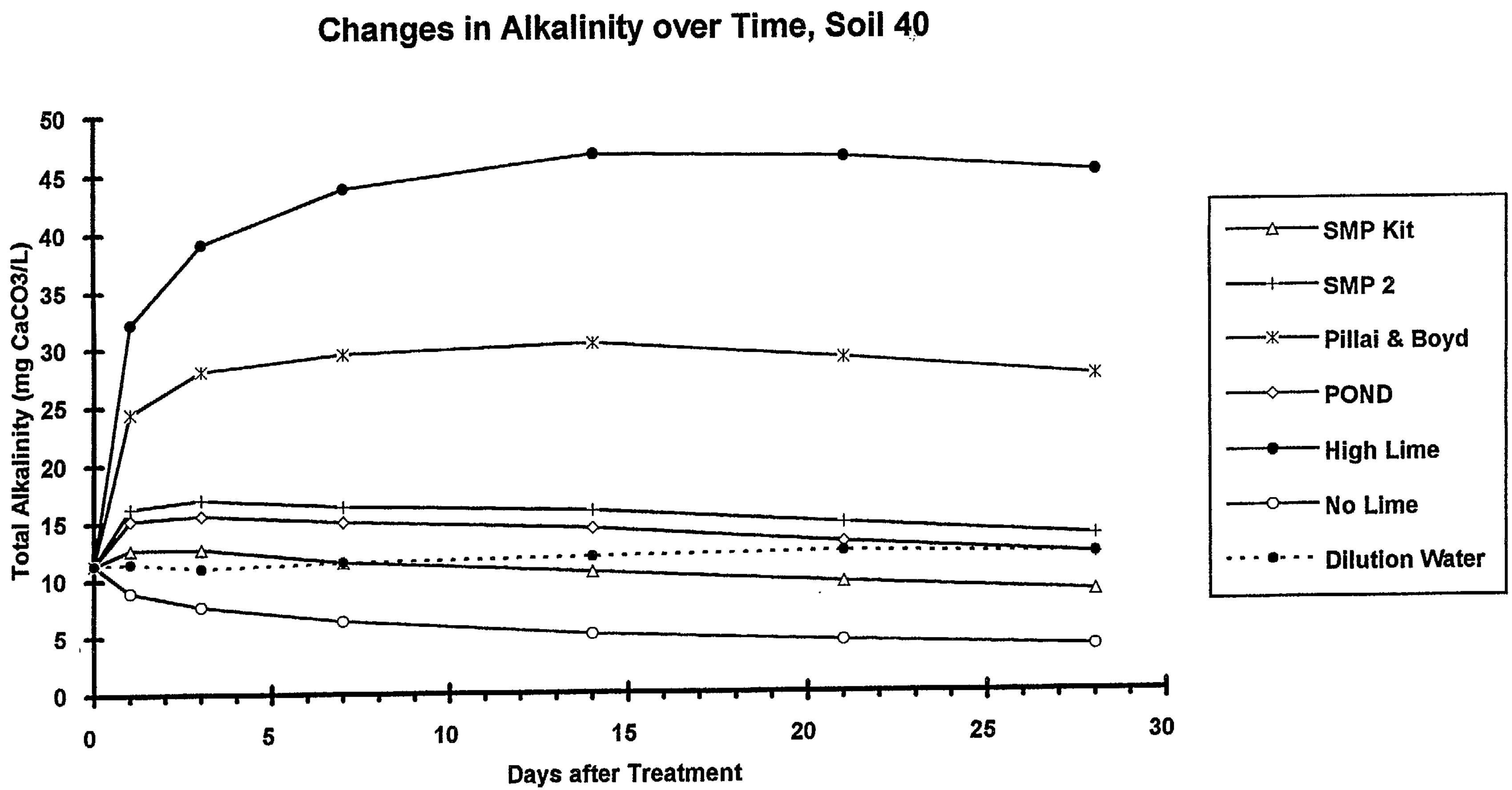

Figure 3. Changes in alkalinity over time for microcosms containing Soil 40. Only the Pillay and Boyd lime requirement estimator provided adequate levels of alkalinity in microcosms containing this soil.

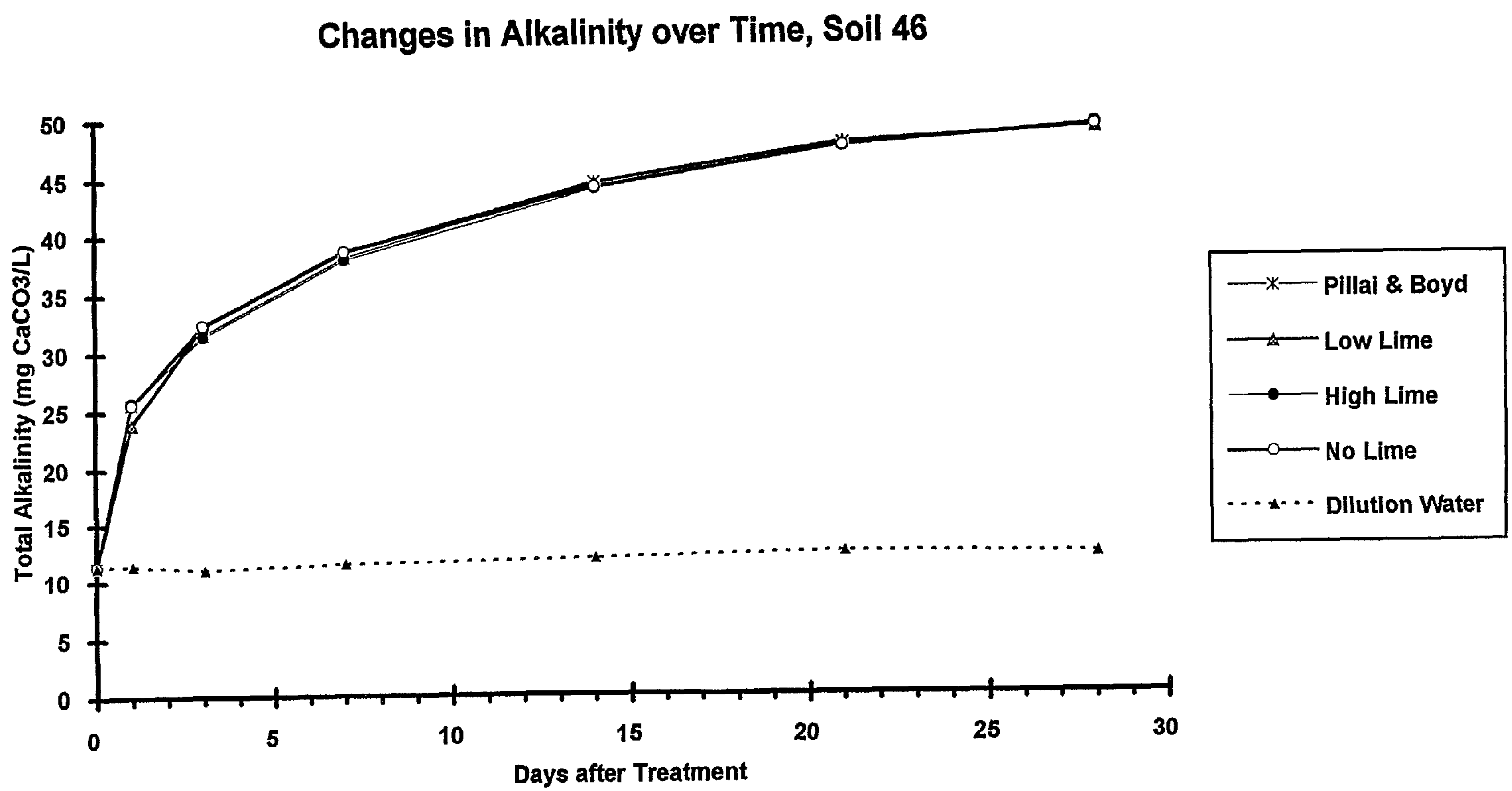

Figure 4. Changes in alkalinity over time for microcosms containing Soil 46. The total akalinity in all microcosms containing this soil rose throughout the experiment, apparently approaching an asymptotic level of about 50mg/L as $CaCO_3$. This pattern was the same regardless of whether lime was applied or not, and regardless of how much lime was applied.

Results and Discussion

The laboratory work for this study has been completed, and data are currently being analyzed. Plots of the changes in total alkalinity (mean values for each treatment) for four soils treated according to lime requirement estimates obtained by different methods are shown in Figs. 1 to 4. Preliminary analysis of these changes does not reveal consistent performances of the various methods of estimation across soil types, suggesting different estimators perform better with specific soil types. (Figs. 1, 2, and 3). The total alkalinity patterns in microcosms containing Soil 46, an alkaline soil (pH >7.8) with no lime requirement, except by the method of Pillay and Boyd (1985), were the same regardless of whether lime was applied or not, and regardless of the amount of lime applied.

Anticipated Benefits

The most suitable lime requirement estimator in terms of producing correct alkalinity responses can ensure effective and cost efficient lime applications to users. Where estimations are equivalent, the opportunity to select convenient and inexpensive estimators can extend the practical use of these tests. Efforts are underway to identify the most suitable lime requirement estimators for particular pond soil types through a more complete analysis of the data from these experiments.

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Experimental Evaluation of Lime Requirement Estimators for Global Sites-Isolation Column Experiment

Interim Work Plan, Africa Study 3

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(Printed as Submitted)

Introduction

Aquaculture ponds with acid bottom muds and soft waters are commonly treated with lime to raise soil pH and base saturation levels and to increase the alkalinity of the pond water to an acceptable level. Pond mud pH readings of less than about 6.0 or pond water alkalinities of 20 mg $CaCO_3$/L or less are usually taken as indications that a given pond needs to be limed (Boyd 1979). Aquaculturists have used a number of methods (both agricultural and aquacultural) to estimate the amount of lime that should be added to ponds. Agricultural methods generally estimate the lime requirement (LR) for raising soil pH to a particular level, whereas aquacultural methods go a step further by estimating the LR for raising pond water alkalinity to a desired level.

Study B of Revised Work Plan 7 was designed to evaluate the suitability of several different LR estimators for different types of soils by testing them in laboratory microcosms. This extension of that study was designed to investigate the use of artificial enclosures ("isolation columns") as in-pond test units, and to compare the results obtained in such enclosures with results obtained in laboratory microcosms.

Materials and Methods

Soil samples from a pond with acid soil and low alkalinity water at Soap Creek (Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon, USA) were collected, air-dried, and crushed to pass a 2mm sieve, and the lime requirement of a composite sample was determined according to the method of Pillai and Boyd (1985).

Isolation columns were constructed using 5-gallon plastic buckets with the bottoms cut out, 29-cm diameter "layflat" polyethylene tubing, rigid 30-cm steel rings, plastic clothespins, and 6-ft fiberglass plant stakes. Six of these columns were pressed firmly into the pond bottom (minimum depth of 10cm into the soil) in a single pond at Soap Creek. The columns were placed at a water depth of approximately 1m. Two treatments were applied to the columns in triplicate: three columns were limed according to the Pillai and Boyd (1985) estimate, and three columns were left unlimed. Water column samples (from the surface to approximately 10cm above the pond bottom) were collected after 1, 3, 7, 14, 21, and 28 days and returned to the laboratory for immediate determination of total alkalinity. Samples were taken at approximately 1100 hours on each sampling day. The starting, ending, and sampling dates for the isolation column portion of the experiment were the same as for the laboratory part.

The laboratory microcosm part of the experiment was conducted in a constant temperature room at the Oak Creek Laboratory of Biology, Oregon State University, Corvallis, Oregon. Glass beakers with a capacity of 800 ml were filled with 750 ml of soft dilution water (alkalinity of approximately 18 mg $CaCO_3$/L, to nearly match the alkalinity in the pond at Soap Creek). Subsamples of the composite soil sample from the pond at Soap Creek were used in laboratory microcosms. The appropriate amount of agricultural limestone was thoroughly mixed with 25 g of the soil and then added to the dilution water. The soil-lime-water mixture was stirred vigorously with a glass rod for 10 seconds to begin the experiment. An unlimed treatment was also prepared. Each treatment was applied in triplicate. Water temperatures in the beakers were maintained at between 23 and 26°C. Samples of approximately

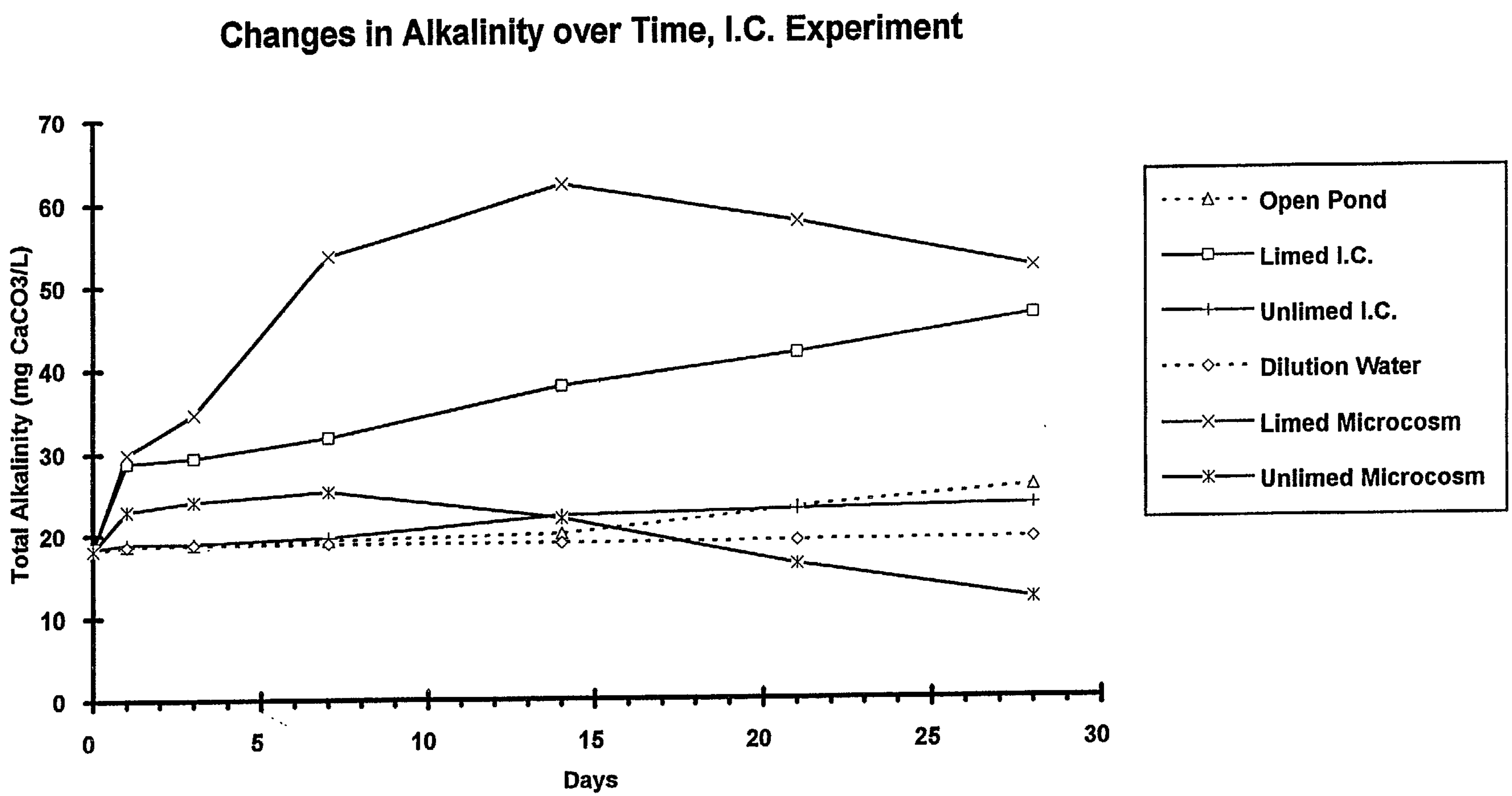

Figure 1. Trends in total alkalinity for in-pond enclosures (isolation columns), open pond water, and limed and unlimed laboratory microcosms containing soil from the same pond. Isolation columns and microcosm treated identically did not respond in the same way, whether limed or unlimed. The alkalinity trend in unlimed isolation columns was similar to that in the open pond.

12.5 ml were removed after 1, 3, 7, 14, 21, and 28 days for determination of total alkalinity. Alkalinity was determined according to the methods described in Standard Methods (APHA, 1989).

Results and Discussion

The field and laboratory portions of this study have been completed and data analysis is in progress. A plot of the total mean alkalinity trends for each treatment for samples taken from the Soap Creek open pond, limed isolation columns, and unlimed isolation columns and the laboratory microcosms (limed and unlimed) is shown in Fig. 1. Preliminary analysis of these trends shows that the responses of laboratory microcosms and in-pond enclosures were similar after 28 days although the time course of alkalinity was not the same for either unlimed treatments or treatments limed at the same rate (Fig. 1). Total alkalinities in the unlimed isolation columns remained close to those in the open pond, suggesting that under the conditions of this experiment the effect of the isolation columns on pond water alkalinity may have been minor. This suggests the small laboratory microcosms do not represent pond responses as well as isolation columns for unlimed soils.

Anticipated Benefits

The suitability of in-pond enclosures of the type tested for conducting liming and possibly other chemical experiments will be determined. Comparison of the results from the enclosures with results from laboratory microcosms will allow additional evaluation of the results of microcosm experiments conducted in the laboratory.

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Problem Perceptions, Production Practices, and Economic Incentives for Tilapia Producers in Four PD/A CRSP Countries

Work Plan 7, Socioeconomic Study

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Introduction

Much of the work of the PD/A CRSP has been directed to specifying optimum ways farmers can fertilize their ponds to increase fish yields. The PD/A CRSP program has identified many of the needed parameters that apply across diverse environments. As a synopsis of the three main aspects of a larger study conducted under the aegis of the Pond Dynamics/Aquaculture CRSP, this article summarizes the main findings of a larger report that establishes how and to what extent the research processes are affecting institutions serving farmers in PD/A CRSP countries and whether they in turn are better able to influence fish farming practices. An economic analysis of experiments featuring various combinations of inputs made in wet and dry seasons are presented. The primary contours of farmer practices and perception related to feeding, fertilizing, and marketing tilapia are summarized. The institutional connections of the PD/A CRSP to universities, government agencies, private industry, and grassroots organizations are many and complex. For numerical results and detailed analyses of these data, the reader is referred to the more comprehensive technical publication that documents this work (Molnar, Hanson, and Lovshin 1995).

Method

Economic Analysis

One objective of this study was to determine the costs and returns associated with alternative production regimes specified by the PD/A CRSP workplan to establish a baseline profile of financial profitability per system per country. The economic analysis utilizes the survey data and other information obtained from PD/A CRSP publications, interviews with participating scientists, and others to examine the economic viability of various experimental outcomes associated with several years of parallel experimentation. Economic viability was assessed with primary data obtained from PD/A CRSP scientists according to their 1983-92 workplan and nutrient input regime testing.

Farmer Surveys

Interviews were conducted with tilapia farmers in four PD/A CRSP countries; Rwanda, Honduras, Thailand, and the Philippines. In Rwanda, 21 active Rwanda fish farmers in eight local administrative districts (communes) were interviewed in the Kinyarwanda language during the Winter and early Spring of 1992. Data were obtained in Spanish from a sample of 51 active Honduran fish farmers in nine of 15 Honduras departments during the Fall 1993. Data were obtained from a sample of Philippine fish farmers in four of 15 provinces on the main island of Luzon during Winter 1994. The survey was revised and adapted in English; some interviews were conducted in the Tagalog language. Data were obtained from a sample of 51 active Thai fish farmers in three of 75 Thai provinces during Winter 1994. All interviews were conducted in Thai.

Institutional Analysis

The institutional connections of the PD/A CRSP were profiled using information obtained in published documents and from interviews and other fieldwork conducted during visits to each country. Based on information obtained from PD/A CRSP scientists, host country counterparts, and other knowledgeables, the institutional context and connections of the research program is portrayed. Though only the main findings are summarized here, the larger report details the main pathways of information exchange and direction that connect the PD/A CRSP to larger organizational systems and the farm level.

Results

Economic Analysis

The economic analysis portrays the relative profitability of various combinations of feeds, fertilizers, and production strategies. Of central interest are the trials with the highest production and those with the highest profitability; they are often not the same.

Rwanda PD/A CRSP Cycle I experiments included chemical fertilization and triple superphosphate (TSP) trials during the dry and wet seasons. There were no Cycle II experiments. In Rwanda, Cycle III experiments included dried chicken manure (CM) treatments during the dry and wet seasons. In nearly every case, dry season yields exceed wet season yields. Dry season production which increased partially because the wet season trials which improved the pond soils by partially buffering the heavy clay soils of these newly constructed ponds. Partial net returns revealed that chemical and organic fertilization to be economical, but both were difficult to obtain or redirect from crop agriculture toward aquaculture.

Honduras CRSP Cycle I research included TSP trials during the dry and wet seasons. Cycle II trials included using chicken manure, cow manure and urea-TSP combinations during the dry and wet seasons. Cycle III trials used layer chicken manure at 125, 250, 500, and 1,000 kg/ha/wk rates during the dry and wet seasons. The highest partial net returns resulted from the use of chicken manure at 1,000 kg/ha/week in the dry and wet seasons. There was no apparent seasonal effect. The 500 kg/ha/wk CM treatment rate similarly had no seasonal effect. However, the next highest production and partial net return was obtained by the 575 kg/ha/wk CM treatment during the dry season, but the same treatment for the wet season had much lower production and returns. When comparing TSP alone or TSP plus urea, the combination of urea and TSP increased production and profitability.

Philippine PD/A CRSP Cycle I research included TSP trials during the dry and wet seasons. Cycle II trials included a) no feeding or b) feeding with supplemental chicken manure or c) feeding with supplemental inorganic fertilizer (16-20-0). Wet season chicken manure fertilization trials were conducted at 125, 250, 500, and 1,000 kg/ha/wk rates. No dry season replication of these treatments occurred. Dry and wet season TSP trials and wet season trials varying chicken manure produced good yields, and were viable financially. Treatments combining chicken manure with inorganic fertilizer (16-20-0) were also economically viable in both seasons. However, when feed was added to chicken manure, negative partial net returns resulted.

When triple inorganic fertilizer (16-20-0) was added to a schedule of feed and chicken manure, production soared above all other nutrient regime treatments. Nonetheless, three out of five treatments resulted in negative net return. The negative returns are caused by the high cost of commercial feeds which is not balanced by sufficient additional income from the additional fish produced. The highest yields were not the most economically viable.

Thailand PD/A CRSP Cycle I experiments used inorganic TSP fertilizer at 8 kg/ha/month during the dry and wet seasons. Cycle II experiments included trials using chicken manure and urea plus TSP during the dry and wet seasons. Cycle III research included chicken manure trials during the dry and wet seasons. The economically viable enterprises involved only chicken manure. Production was good for all chicken litter treatments. However, no treatments using TSP alone or with urea were economically viable. The low price paid for tilapia in Thailand and relatively higher cost of chemical fertilizers in relation to chicken manure led the partial net returns for the chemical fertilizer treatments to be negative even though production was good. The treatment with the highest production is not the most profitable here.

Survey Findings

Fish Feeding

Farmers in the four countries fed their tilapia a variety of different items reflecting differences in the intensity of aquaculture practice in each nation. Feeding and fertilization represent overlapping activities for the tilapia farmer; unconsumed feed fertilizes the pond water and some part of organic inputs are directly consumed by the fish. In Rwanda, respondents primarily understood questions about feeding in terms of the amount and kind of organic materials they put in their ponds. In the other sites, farmers primarily understood feeding to refer to the use of commercial, purchased feeds.

Commercial feed was not used in Rwanda; two-thirds of the Hondurans did not use commercial feed; and about half the Philippine respondents did not use commercial feed. Thai farmers were most dependent on commercial inputs to raise their tilapia crops. They also used the most diverse variety of feeds, reflecting the high level of availability of different feed types and a greater willingness to use feeds for other animals for the fish as well.

Fertilization

In Rwanda, commercial fertilizer represents a cash outlay that subsistence farmers prefer to avoid and is generally not applied to fish ponds. Hondurans typically use cattle and chicken manure as fertilizer for their ponds. Chicken manure is the most frequent pond fertilizer in Thailand and the Philippines. Many Rwandan farmers indicated a passive approach to fish farming, as only about half said the ponds were visited every day. Philippine farmers spent the most time with their ponds when they visited them; Thai farmers the least.

Fingerlings

Rwandan farmers are dependent on government hatcheries for fingerlings, although farmers frequently sell fingerlings to one another. Similarly, few private fingerling dealers have evolved in Honduras. The private sector provided fingerlings to more than 80 percent of the Thai farmers and about 37 percent of the Philippine operators. In each country, most farmers were using the *Oreochromis niloticus* species. Thai and Philippine farmers tend to densely stock the smallest fingerlings available. Honduran farmers tended to stock somewhat larger fingerlings. All-male tilapia were stocked in each country, although Rwanda tended toward more mixed-sex production.

Stocking and Grow-Out Practices

Most farmers are growing but a single crop of tilapia each year in Rwanda and Thailand. In Honduras, almost half reported two or more crops, but in the Philippines two-thirds obtained two crops per year.

In Rwanda, cooler water and poor quality inputs slow fish growth and lengthen the crop cycle to eight months or more. Warmer water in Honduras allowed more than a quarter of the sample to report growing tilapia in less than 180 days. Polyculture, or raising more than one species of fish in the same pond, was practiced by nearly all the Thai farmers in the study.

Marketing Problems

Philippine farmers indicated no trouble marketing their fish. Marketing difficulties of some kind were reported by about a third of the Thai respondents, and around 20 percent of the Honduran and Rwandan respondents. Over half the Thai farmers reported difficulties securing the price they wanted for their tilapia. Honduran farmers were the most confident about being able to sell their tilapia at some price, even if it was not what they originally asked.

About a third of the Rwanda sample said that there were many people in their area that did not like tilapia. Around 15 percent of the Honduras and Thai respondents felt this way, but no Philippine respondent said so. Of the four countries, the Philippines seems to have the highest consumer acceptance of tilapia.

Impacts on Households

About 78 percent of the Philippine farmers thought that there were points in the annual farm cycle when the pond was too much work, 40 percent said so in Thailand. Few of the other respondents thought so. Previous work suggests that Rwandan women are much more likely to report these difficulties. About 80 percent of the Philippine, Thailand, and Rwanda respondents felt that tilapia fit well with other farm activities, but only 64 percent of the Honduran farmers thought so.

Three quarters or more of the respondents in the Philippines, Honduras, and Thailand noted the benefits of additional cash for their households as something associated with the tilapia crop. Only 5 percent of the Rwandans agreed with this statement, as the limited amount of cash produced by tilapia tended to be used mainly by men for other purposes.

Pond Conflicts

Thai farmers were most likely to note problems over water resources emanating from the tilapia crop (57 percent), an issue noted by only a few of the other respondents. Philippine operators had few problems with predators eating their fish, but this was an issue for farmers in each of the other countries. Theft was a concern for 44 percent of the Honduran farmers, but only 20 percent or so of the other respondents noted this as an issue; 11 percent in the Philippines. Thai farmers were most likely to agree that tilapia were easier to steal, though a third of the Honduran respondents thought so as well.

Prospects for the Pond

Most respondents thought their fish pond produced enough to be worth the work they put into it, though Rwandans were slightly more skeptical. A third of the Hondurans questioned the fit of tilapia with the other activities of their farm household. About 60 percent of the Hondurans thought that tilapia was less profitable than their other activities.

Most respondents thought tilapia was the best use of the land it occupied. Hondurans were more likely to report themselves as planning to build new ponds (39 percent). In land-short Rwanda only 11 percent thought so. Only 54 percent of the Rwandans were happy with tilapia as a type of fish to grow; they desired a larger, faster growing fish even though water temperature and a shortage of quality inputs were the main constraints. More than 90 percent of respondents in the other nations were happy with tilapia as a type of fish to grow. The perceived profitability of tilapia relative to other farm activities was highest in the Philippines, where 90 percent thought it was more profitable than other crops. Overall Hondurans were least happy with the returns from tilapia, though Thai farmers were less convinced that tilapia ponds were the best use of the land. Lowland Thai farmers with irrigation in the far reaches of the Bangkok marketing area have many enterprise choices and marketing opportunities.

Most fish farmers surveyed in Rwanda, Thailand, Philippines and Honduras felt that the tilapia pond was the best use of the land it occupied on their farm. As the Thailand respondent's pond area increased, a smaller percentage replied that the pond was the best use of the land occupied. All Philippine owners, regardless of pond category agreed aquaculture was the best use of the land. All pond size owners in the Philippines felt very positive about aquaculture in relation to other farm activities. In Thailand, small and medium pond size owners shared a similar high degree of enthusiasm about tilapia culture, but only about half the large size pond owners in Thailand agreed that tilapia was more profitable than other farm activities.

Institutional Networks

One common pattern across the four PD/A CRSP sites considered here is the upstream nature of the PD/A CRSP contribution to technology transfer. Although farmer trials have been conducted at one time or another in each site, these efforts largely have been singular or specialized events and not part of a systematic program. In none of the countries do farmers have a regular pattern of contact with a private or governmental technology transfer agent of whatever stripe. What limited efforts are underway tend to have only sporadic and indirect communication ties to PD/A CRSP researchers and host institutions.

Farmers rely heavily on word-of-mouth and a melange of information sources and experiences most of which have little connection to the PD/A CRSP. Most of the farm-level impact of PD/A CRSP activities is second order; that is, PD/A CRSP research information is absorbed, integrated with other messages, and retransmitted by private firms and national institutions. The messages are received by innovator farmers, private managers, hatchery personnel, trainers, consultants, and others who will use the information to make decisions about growing fish. The messages also affect what these individuals tell others who want to or already are raising tilapia.

The most immediate impacts of PD/A CRSP activities are manifested primarily in the training experiences of degree candidates at institutions of higher learning such as the Asian Institute of Technology, University of Rwanda, Zamarono University, or CLSU. PD/A CRSP personnel serve as thesis advisors or consultants for faculty and students conducting aquacultural research or have other ties with these institutions. The insights, paradigms, organizing frameworks, and scientific technique communicated during these activities represent a major technology transfer impact of the PD/A CRSP.

In each country, PD/A CRSP researchers have direct contacts with extension or outreach staff working in fish culture. The collegial relationships, information exchanges, mutual assistance, and other forms of mutual influence also are a means for furthering the influence of PD/A CRSP research. Often diffuse and subtle, but occasionally direct and focused, PD/A CRSP research operations and research findings contribute to the information milieu surrounding each nation's aquaculture industry.

Extension programs and the training of extension personnel are only indirectly influenced by the PD/A CRSP. In the Philippines, village-level extension in aquaculture does not exist. It is at best highly variable in the other nations. Even in Thailand, with the largest and best-developed network of personnel and facilities devoted to fish culture, PD/A CRSP ties to extensionists are infrequent and weak.

The many institutional actors working in aquaculture perhaps should be considered the primary audience for a global research project such as the PD/A CRSP. Although some level of direct farmer contact and training is necessary for keeping PD/A CRSP scientists in touch with the direct experiences and problems of fish farmers, the impacts and influence of the PD/A CRSP may be greater if institutions and industry are understood to be the primary consumers of PD/A CRSP outcomes.

Thus, seminars for NGOs that maintain extensive and long-term relationships with villages and small-scale farmers may be the most important mechanism for reaching this constituency than direct intervention by the PD/A CRSP. As long as small- and medium-scale farmers remain a central target segment for PD/A CRSP research impacts, the development of a continuing network of contacts with representatives of these groups will be a significant objective for the PD/A CRSP. The nongovernmental organizations (NGOs) may be more effective at stimulating interest and reaching small-scale farmers than governmental organizations or the limited and sporadic activities of PD/A CRSP personnel.

To gain greater leverage for PD/A CRSP activities, a number of strategies might be consciously highlighted for PD/A CRSP scientists. These include; training trainers, encouraging NGOs to adopt aquaculture as part of their repertoire of assistance activities, and helping national institutions with seminars and training programs for NGOs. These and other means may be used for wholesaling PD/A CRSP technology to actors closer to village life who will be there when PD/A CRSP is not.

In terms of changes in national aquaculture industries, the impacts of the PD/A CRSP are manifold. The larger report details the institutional context of each nation and portrays the role of the PD/A CRSP in the nation's technical-knowledge system for aquacultural development. Many of the advances that take place in an aquaculture industry are facilitated by the formal and informal consulting of PD/A CRSP scientists with private sector firms that grow fish or manufacture and sell inputs to farmers. The presentations at meetings, visits to laboratories and facilities, and personal communications with industry scientists and managers remain a continuing nexus of impact for the PD/A CRSP.

Conclusion

Tilapia growers in each of the countries face vastly different institutional systems supporting tilapia production. The impacts of the PD/A CRSP are muffled by the inherent characteristics of the research process, the nature of institutional functioning in each country, and the dynamism of the information environment for aquacultural technologies.

The communication process linking experimental pond to farm practice involves several layers of translation and transmission. Many factors interact to affect the extent and degree of impact of PD/A CRSP scientists and research programs on national aquacultural institutions and farm practice. Experimental findings are at base experimental; they reflect controlled conditions and careful measurement of a focused set of factors. Farm conditions reflect variable physical and managerial situations that often mitigate the impact of effects identified by repeated experimental trial. That is, experimental findings often must be cumulated from many studies and modified in certain ways to generate a robust field recommendation. In essence, an internal process of recognition and acceptance must take place within national research and extension systems before the findings become farm-level practice. In some cases, farmers undertake new approaches independently of national systems and scientists and extension personnel learn from innovative farmers.

Where PD/A CRSP activities have the opportunity to influence host country governmental assistance to aquaculture, efforts should emphasize infrastructure and improved functioning of the private sector. Poorly organized fish product markets and input distribution systems often hinder aquaculture development. As markets for tilapia expand, production and support services demand will also expand. Development of private sector marketing services for both production inputs and fish outputs are needed for sustained aquacultural development. Weak connections to the farm level characterize the institutional context in each PD/A CRSP country. Thus efforts to enhance the transfer and utilization of PD/A CRSP research results will require greater attention to actual and potential pathways of influence and information flow to the farm and village. Better understanding of these relationships will facilitate the conduct of a research program that meets farm-level needs in an environmentally and socially sustainable way.

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Decision Support for Pond Aquaculture: Simulation Models and Applications

Work Plan 7, DAST Studies 3, 4 and 5

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Introduction

Simulation models in the decision support system *POND* are organized hierarchically into different levels to enable users to perform different kinds of analyses based on data availability and output resolution requirements. The initial version of *POND* (Version 2.0) provided capabilities for two levels of modelling (Bolte et al., 1995; Piedrahita et al., in review). During the period following the release of *POND* Version 2.0, several modifications were made to the Level 1 and 2 simulation models. The objectives of these modifications were to provide improved methods for examining the effects of different stocking and harvest dates on production, better techniques for predicting feed, fertilizer and water requirements, and capabilities for considering the effects of feed quality on uptake by fish and waste generation within ponds. A more complex, research-oriented level of modelling that considers fundamental processes involved in nutrient dynamics and other issues of water/sediment dynamics has also been implemented in *POND* Version 2.5.

The design philosophy of the *POND* software is such that it can be readily adapted for different species and/or culture conditions (Bolte et al., 1995). In order to demonstrate the applicability of the software for such situations, an effort was undertaken to parameterize the fish bioenergetics for channel catfish (*Ictalurus punctatus*), tambaquí (*Colossoma macropomum*) and pacu (*Piaractus mitrei*). Simultaneous validation of the water temperature model for CRSP and non-CRSP locations was also undertaken. Applications of *POND* for a variety of pond culture scenarios have also been explored.

The POND Modelling Framework

The general framework of *POND* functionality and application areas of the software are indicated in Figure 1. Further details regarding the *POND* software architecture and the object-oriented paradigms used are presented elsewhere (Bolte et al., 1995; Piedrahita et al., in review). Advantages of simulation models for pond aquaculture have also been previously presented (Nath et al., 1995).

Simulation models in *POND* provide opportunity to address fish bioenergetics, water temperature and volume changes, water/sediment quality dynamics, and primary and secondary productivity under different pond conditions. The fish bioenergetics model is documented elsewhere (Bolte et al., 1995). Models used to describe the dynamics of the other components within a pond are based on principles of mass and energy (for water temperature calculations) balance. Details of reactor kinetics are beyond the scope of this discussion and may be found in textbooks for waste water engineering (e.g., Benefield and Randall, 1980) or reactor design (e.g., McDuffie, 1994). However, a brief summary is provided below.

Reactor principles used in POND models

The previous version of *POND* assumed that ponds can be treated as continuously stirred batch reactors with a constant volume and zero flow. The material balance for a state variable in such a reactor is given by:

$$\frac{dC}{dt} V = R_C V \qquad (1)$$

where C = concentration of the material (e.g., g m^{-3}), V = pond volume (m3) and R_c = sum of the source and sink processes affecting the material (e.g., g m^{-3} d^{-1}). Because V is a constant, $dC/dt = R_c$. The assumption of batch reactor kinetics has been removed in *POND* Version 2.5, in which ponds are assumed to be continuously stirred tank reactors (CSTR) with unsteady flow. The differential equation expressing the change in concentration of a state variable in such reactors is given by:

$$\frac{dC}{dt} = \frac{Q_i\ Q_i}{V} - \frac{Q_o\ C_o}{V} + R_c - \frac{C}{V}\frac{dV}{dt} \qquad (2)$$

where Q_i = influent rate (m^3 d^{-1}), Q_o = effluent rate (m^3 d^{-1}), C_i = material concentration in the influent (g m^{-3}), and C_o = material concentration in the effluent (g m^{-3}). Note that Eq. 2 addresses the three typical conditions under which aquaculture ponds are operated or need to be analyzed: (a) ponds with a constant volume (i.e, Q_i, Q_o and $dV/dt = 0$, in which case Eqns. 1 and 2 are identical), (b) ponds without regulated flow (Q_i and Q_o = 0) but in which substantial volume changes may occur due to seepage, evaporation and/or runoff events, and (c) ponds in which flow considerations are important (e.g., shrimp facilities). For the latter two conditions, volume changes may have implications for fertilization and effluent quality management. Use of Eq. 2 in the *POND* framework also implies that the models can be used to analyze fish culture in tanks where flow considerations may be particularly important.

System Processes and Model Organization

Source and sink processes (including regulated flow and non-flow related volume changes) considered at the three modelling levels in *POND* Version 2.5 are listed in Table 1. A detailed listing of the equations used to describe system dynamics is available from the authors. Each of the modelling levels are briefly described below.

Level 1

Level 1 models are relatively simple and require minimal data inputs. Pond volume has been added to the list of state variables previously maintained (i.e., fish mass and water temperature) (Fig. 2; see also Table 1). Weather data that are required for the latter two variables may either be generated by a simple weather generator embedded in *POND* or read from ASCII files provided by the user. Use of the pond volume model is optional; if it is not used, ponds are assumed to have a constant volume. This model has been adapted from Yoo and Boyd (1994) and provides capabilities for considering regulated flow, watershed runoff, seepage, evaporation and overflow. Optionally, the volume model can be used to estimate the amount of water required to maintain a desired pond depth. The addition of flow considerations to the *POND* framework has also necessitated the addition of a new database to the software (Fig. 1) that enables definition of source water quality characteristics.

At Level 1, fertilization rates and supplementary feeding schedules are either generated by *POND* or specified by the user. In order to predict fertilization rates for long-term simulation periods (e.g., one or more seasons), a modified approach of the *PONDCLASS* fertilization method (Lannan, 1993) is currently being tested at Level 1. Use of this approach requires a maximum potential light-limited gross primary productivity (GPP) value for the location to be specified. It is also necessary to specify the desired concentration of nitrogen and phosphorus in the water column. Nutrient (i.e., dissolved inorganic carbon, nitrogen and phosphorus) uptake is assumed to follow Michelis-Menten kinetics. The actual GPP level possible in a pond is scaled by the factor (temperature, carbon, nitrogen or phosphorus) that is most limiting to phytoplankton growth. Because this level of modelling does not explicitly account for nutrient transformation processes, it is necessary to approximate such sources and sinks so as to ensure that predicted fertilization rates are consistent with the results of field trials. This is accomplished by defining parameters for various processes (e.g., phytoplankton nutrient recycling via respiration and death, nutrient sinks in fish and sediment-water column exchange).

Feed options available in *POND* Version 2.5 include the ability to specify whether fish lots feed only on natural food resources (a function of the critical standing crop; see Bolte et al., 1995), artificial feed, or a combination of these two resources. Artificial feed requirements may be either generated by use of the fish energetics model and a user-defined satiation (target) feeding level or specified from a file in terms of % body weight. A feed database is also available in the new version of *POND* and users can specify the type of feed used. This database enables definition of a coefficient q (0-1), which can be

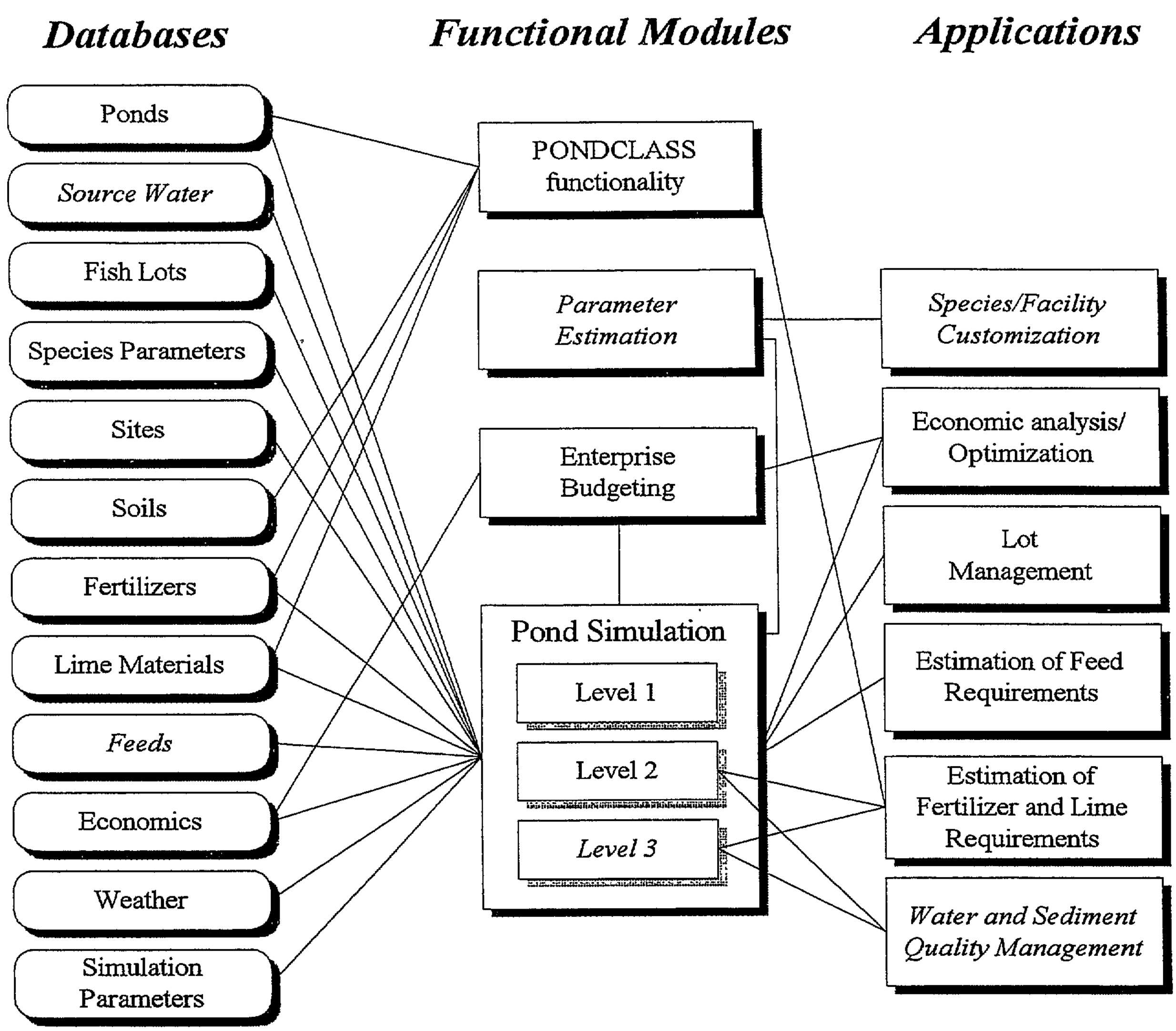

Figure 1. Architecture of *POND* indicating databases, functional modules, and applications for decision support. Italicized items reflect additions to the software that have occurred since the release of Version 2.0.

Table 1. A summary of state variables maintained in *POND* and the source/link processes considered at the three modelling levels. Processes directly manipulated by management practices are italicized. For some variables (e.g., nitrogen, phosphorus), mass balances may vary depending on the modelling level, and separate state variables are maintained for 'sub-components' (e.g., total ammonia-nitrogen, organic nitrogen, etc.). In such cases, the table lists processes that may be considered at all the modelling levels, as well as the additional ones (e.g., atmospheric diffusion of ammonia-nitrogen) specifically affecting the sub-component.

STATE VARIABLE	MODEL LEVEL	SOURCES	SINKS
Fish Mass	1, 2, 3	Natural food uptake *Artificial feed uptake*	Feeding catabolism Fasting catabolism
Water Temperature	1, 2, 3	Net short-wave solar radiation Net long-wave atmospheric radiation *Influent heat*	Long-wave back radiation Evaporative heat loss Conductive heat transfer[a] Non-flow related volume changes[a] *Effluent heat*
Water Volume	1, 2, 3	*Influent water* Direct precipitation Runoff	*Effluent water* Evaporative water loss Seepage[a] Overflow
Water-column Nitrogen (N)	1, 2, 3	*Influent water*	*Effluent discharge*
Total-N	2, 3[b]	Fish respiration + excretion	Non-flow related volume changes[a]
Dissolved inorganic-N	1	Wasted feed Phytoplankton respiration + death Zooplankton respiration + death Bacterial respiration + death *Fertilization*	Phytoplankton uptake Bacterial uptake Sediment sinks/sources[a] Miscellaneous sinks/sources[a]
Total Ammonia-N	3	Ammonification	Nitrification Volatilization[a]
Nitrate-N	3	Nitrification	
Organic-N	3		Ammonification
Water-column Phosphorus (P)	1, 2, 3	*Influent water*	*Effluent discharge*
Total-P	2, 3[b]	Fish respiration + excretion	Non-flow related volume changes[a]
Dissolved inorganic-P	1	Wasted feed Phytoplankton respiration + death Zooplankton respiration + death Bacterial respiration + death *Fertilization*	Phytoplankton uptake Bacterial uptake Sediment sinks/sources[a] Miscellaneous sinks/sources[a]
Inorganic-P	3	Mineralization	
Organic-P	3		Mineralization
Water-column Carbon (C)	1, 2, 3	*Influent water*	*Effluent discharge*
Total-C	2	Fish respiration + excretion	Non-flow related volume changes[a]
Organic-C	3	Wasted feed Phytoplankton respiration + death Zooplankton respiration + death Bacterial respiration + death *Fertilization*	Phytoplankton uptake Bacterial uptake Sediment sinks/sources[a] Miscellaneous sinks/sources[a]
Dissolved Inorganic-C	3	Sediment respiration	Atmospheric diffusion[a]
Sediment-N	2, 3	Supply of water column material:	Water-column sinks/sources[a]
Sediment Total-N	2, 3[b]	From fish excretion, wasted feed, and phytoplankton, zooplankton and bacterial death.	
Sediment Ammonia-N	3	Ammonification	Nitrification
Sediment Nitrate-N	3	Nitrification	
Sediment Organic-N	3		Ammonification

[a] Can be either a source or a sink.

[b] Calculated from concentrations of inorganic and organic forms at Level 3.

Table 1. Contd.

STATE VARIABLE	MODEL LEVEL	SOURCES	SINKS
Sediment-P Total-P Sediment Inorganic-P Sediment Organic-P	2, 3 2, 3[b] 3 3	Supply of water column material: From fish excretion, wasted feed, and phytoplankton, zooplankton and bacterial death. Mineralization	Water-column sinks/sources[a] Mineralization
Sediment-C Total-C Organic-C	2, 3 2 3	Supply of water column material: From fish excretion, wasted feed, and phytoplankton, zooplankton and bacterial death.	Water-column sinks/sources[a] Sediment respiration
Alkalinity	3[c]	*Influent water* Phytoplankton uptake of nitrate-N Ammonification *Lime addition*	*Effluent discharge* Non-flow related volume changes[a] Nitrification Bicarbonate uptake Hydrogen ion production Phytoplankton uptake of bicarbonate Phytoplankton uptake of ammonium ion
Dissolved oxygen	3	*Influent water* Phytoplankton production *Aeration*[d]	*Effluent discharge* Non-flow related volume changes[a] Fish Respiration Phytoplankton respiration Zooplankton respiration Bacterial respiration Sediment respiration BOD of organic fertilizers Atmospheric diffusion[a]

[a] Can be either a source or a sink.
[b] Calculated from concentrations of inorganic and organic forms at Level 3.
[c] Assumed to remain at steady-state for Level 1 and 2 models.
[d] Not currently supported.

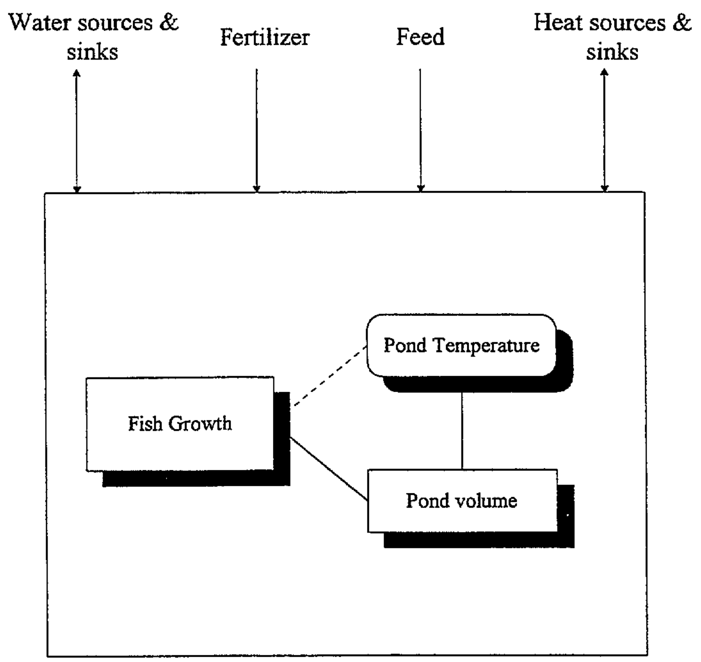

Figure 2. State variables and system inputs/outputs for Level 1 modelling in *POND*.

interpreted as being a measure of both the quality of the supplemental feed as well as feeding practices. For example, feeds like rice bran may not be particularly palatable to some fish species and consequently a large proportion is not directly consumed. A lower value for q should be used in such cases. Conversely, a high quality feed combined with proper feeding practices may warrant the use of a higher value for q.

POND Version 2.5 has also been modified to enable the simulation of multiple fish lots over one or more years of production. Thus, users can specify stocking and harvest dates for each lot, and the software will automatically add and remove lots from a pond as the simulation proceeds. This option is also useful to compare fish yields obtained by stocking and harvesting at different times during a year, particularly for locations with pronounced seasonal differences.

Level 2

Models at this level allow prediction of phytoplankton, zooplankton and nutrient dynamics (total carbon, nitrogen and phosphorus) in the pond water, besides all the functionality of Level 1 (Fig. 3; see also Table 1). In addition, steady state bacterial concentrations are maintained, and nutrient exchange between pond water and sediments is assumed to depend on the concentration gradient between these two components. This necessitates additional state variables for sediment nutrient concentrations. Fertilizer, feed and water requirements may be user-specified or optionally generated by the model.

Level 3

Level 3 models inherit all the functionality of Level 2, and provide additional capabilities for simulating bacterial kinetics, and detailed pond water/sediment quality dynamics (Fig. 4; see also Table 1). Additional state variables for pond water include dissolved oxygen and alkalinity. Further, state variables are also maintained for organic, ammonia and nitrate nitrogen, as well as organic and inorganic phosphorus and carbon in both pond water and sediments. User-specified fertilization and feeding regimes, coupled to pond process-based nutrient mass balances, are used to estimate nutrient consumption and production rates.

Model Calibration and Validation

The *POND* models have been calibrated and validated by the use of data from CRSP and non-CRSP sources. Simulation results for Level 1 fish growth, water temperature and nutrient models are presented below. A constant pond volume was assumed in all cases (i.e., the water balance model was not used) because the experiments were conducted in ponds with more or less constant depths.

Fish Growth

Simulation results for the fish bioenergetics model in *POND* relevant to Nile tilapia growth at different CRSP sites have previously been reported (Bolte et al., 1995). The bioenergetics model used at Level 1 has also been calibrated for channel catfish, tambaquí and pacu by the use of a non-linear, adaptive search algorithm (Bolte and Nath, 1995). Predicted fish growth profiles for these species are compared to observed data in Fig. 5. For these simulations, water temperature values as reported in the data sources were used (see Bolte and Nath, 1995 for details). Simulated fish weights for catfish are very similar to observed data.

The predicted weight for tambaquí during the initial culture period is also very similar to observed data (Fig. 5); however, weight during the exponential growth phase of this species showed some deviation from observed values, although the final weights are very comparable to observed data. Steady state growth rates during the final two months of culture were apparently caused by unseasonably low water temperatures (Merola and Pagan-Font, 1988). Predicted fish weights for pacu are comparable to observed data, except for the last data point when this species appeared to be entering into a very rapid growth phase.

Model calibration results suggest that the energetics model is likely to provide adequate accuracy for most applications (e.g., seasonal yield projections). However, it is possible that one set of model parameters may not be suitable for all growth phases, especially for species (e.g., tambaquí and pacu) which enter into a very rapid growth phase when temperatures are optimal. In such situations or when more accurate predictions are desirable, it may be advisable to separately parameterize the model for the growth of fingerlings and food fish.

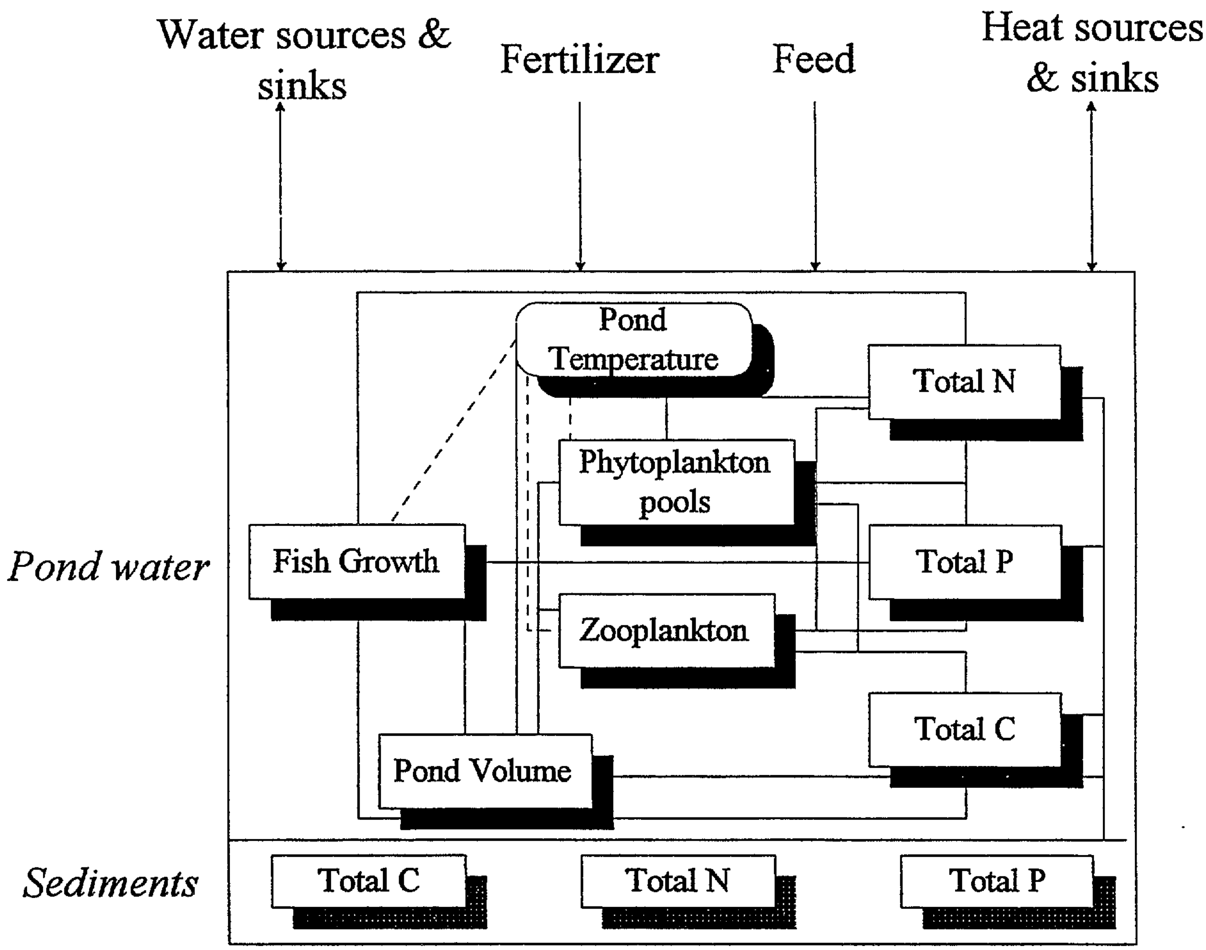

Figure 3. State variables and system inputs/outputs for Level 2 modelling in *POND*.

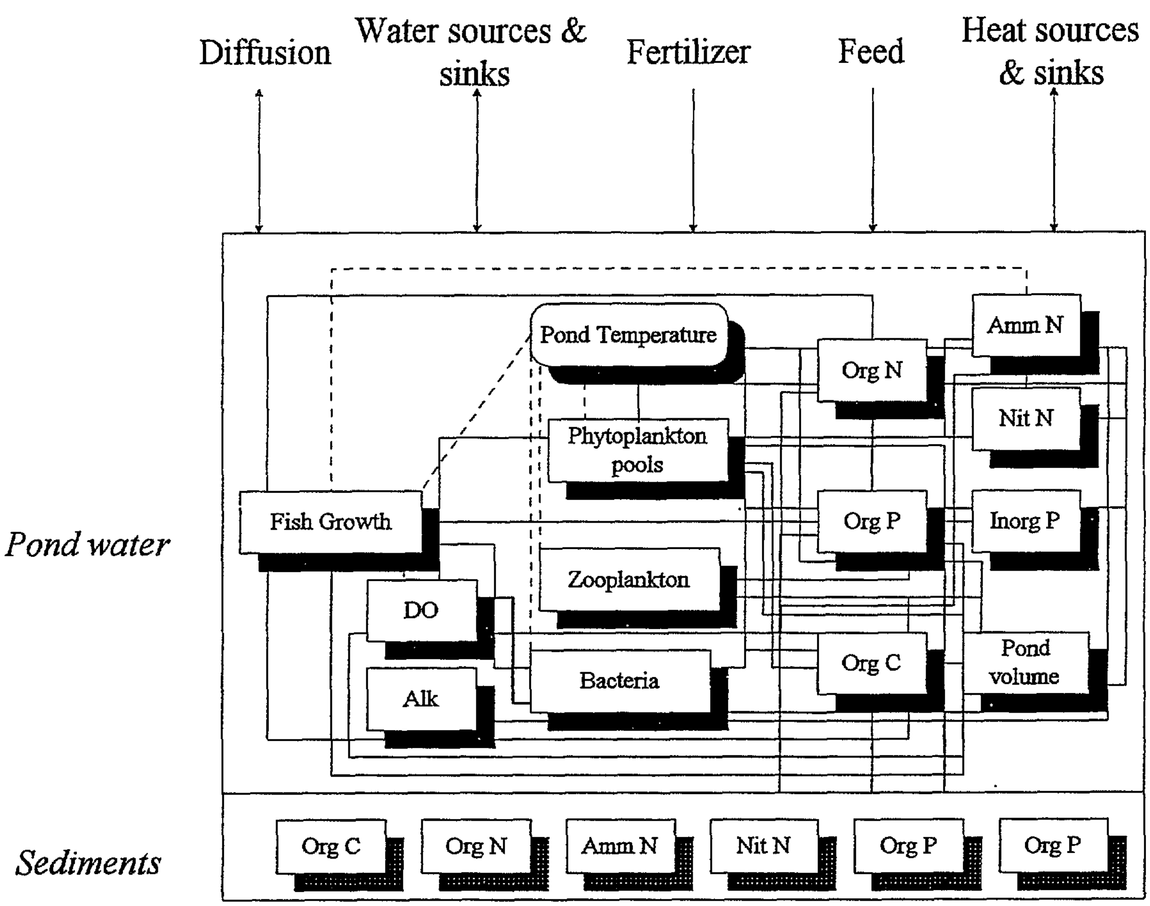

Figure 4. State variables and system inputs/outputs for Level 3 modelling in *POND*.

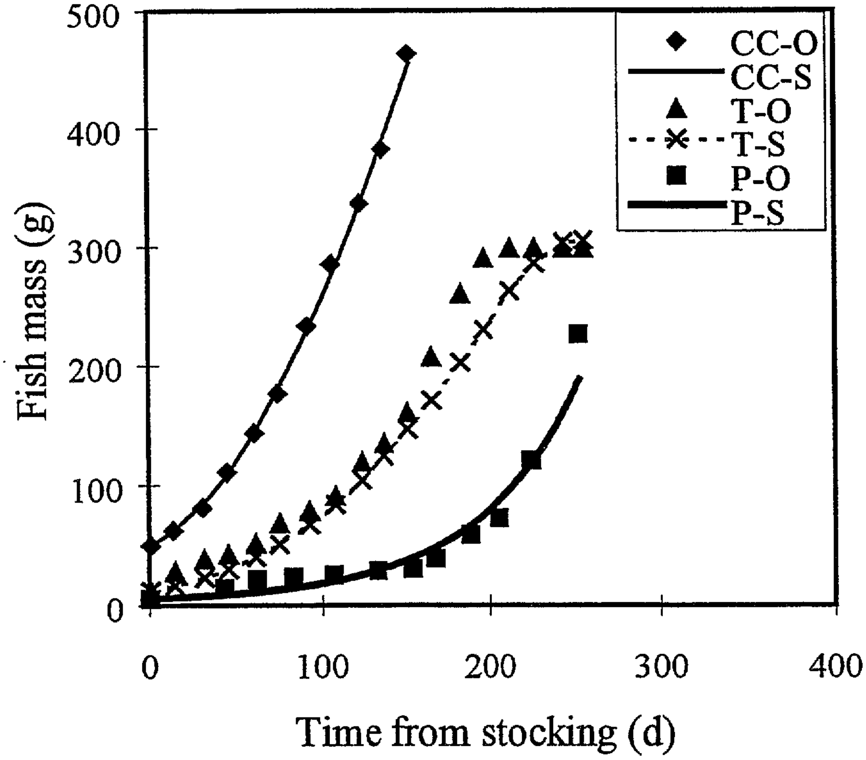

Figure 5. Simulated (S) and observed (O) fish growth profiles for channel catfish (CC), tambaquí (T), and pacu (P).

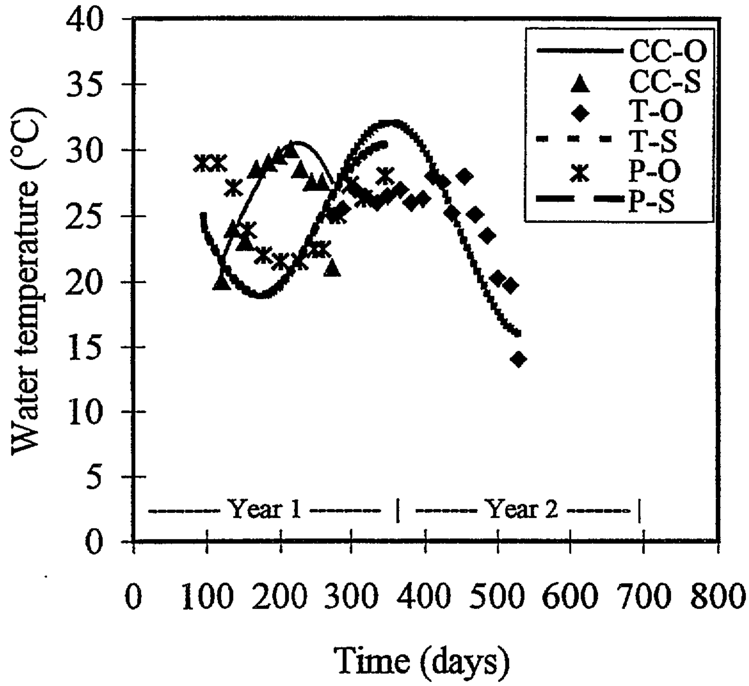

Figure 6. Simulated (S) and observed (O) water temperature profiles for a catfish (CC) pond in Mississippi, and tambaquí (T), and pacu (P) ponds in Brazil.

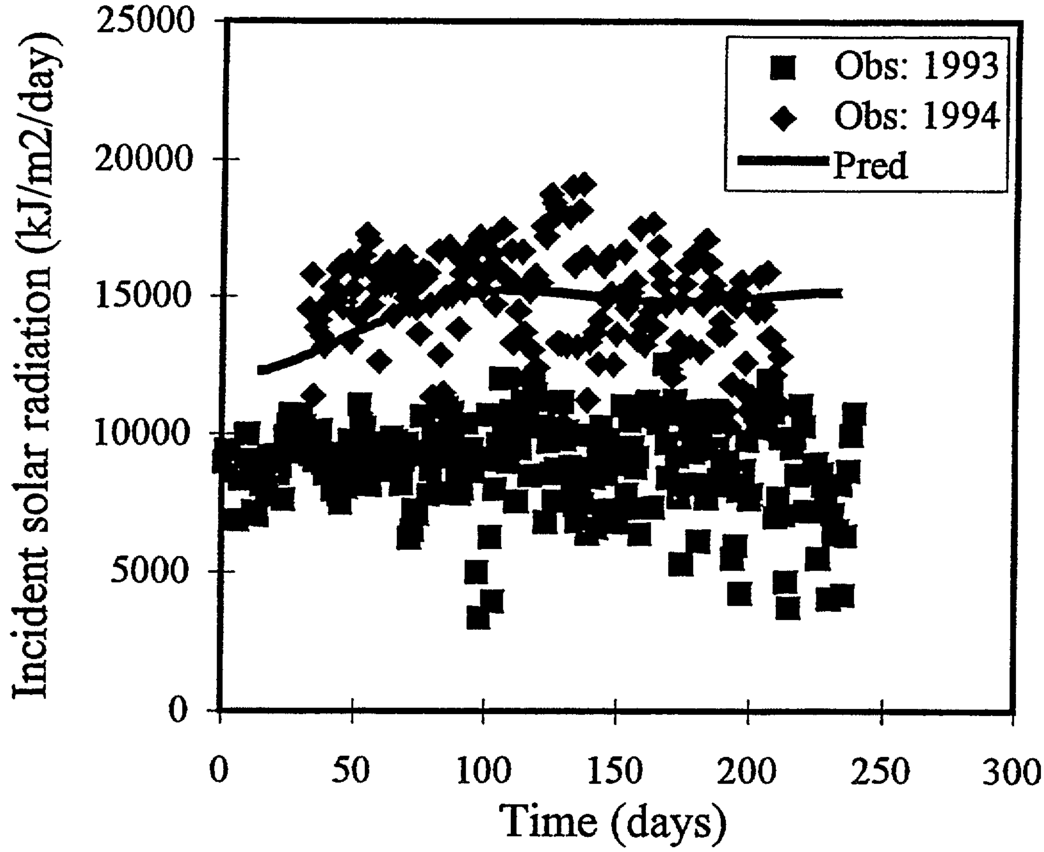

Figure 7. Incident short-wave solar radiation predicted by the use of the weather model in *POND* compared to observed data (recorded in 1993 and 1994) for Bang Sai site. Simulation assumed broken cloud conditions with cloud cover set to 0.75.

Water temperature

Pond water temperature for the sites where the above experiments were conducted was also predicted by the use of the weather generator available within *POND* (Fig. 6). Predicted and observed temperatures for the catfish ponds are similar except towards the end of the simulation period, when observed data were 4-5°C lower than predicted values. For the tambaquí pond, the same trend can be observed except towards the end of the simulation period. Predicted water temperatures for the pacu pond have the same pattern as observed data, but the curve appears to have a distinct lag. This may be due to inaccuracies in the air temperature or solar radiation predictive models (described in Nath and Lannan, 1993); alternately, it is possible that the weather pattern for the year of simulation (1986) differs from the "typical" trend for this location.

Comparisons between predicted and observed weather data used in water temperature calculations (i.e., short-wave solar radiation and ambient air temperature) were undertaken for three CRSP sites: Bang Sai (Thailand), El Carao (Honduras), and Rwasave (Rwanda) (Figs. 7-12). Incident short-wave solar radiation data are not routinely reported in the PD/A CRSP database. However, daily photosynthetically active radiation data (wavelength 400-700 nm; E m^{-2} d^{-1} are reported. These data were converted into short-wave solar radiation values (kJ m^{-2} d^{-1}) by multiplication with a factor of 505.67, which is based on the assumption that 1 E≈ 217.44 J of energy, and that only 43% of the overall radiation is photosynthetically active (Withrow and Withrow, 1956).

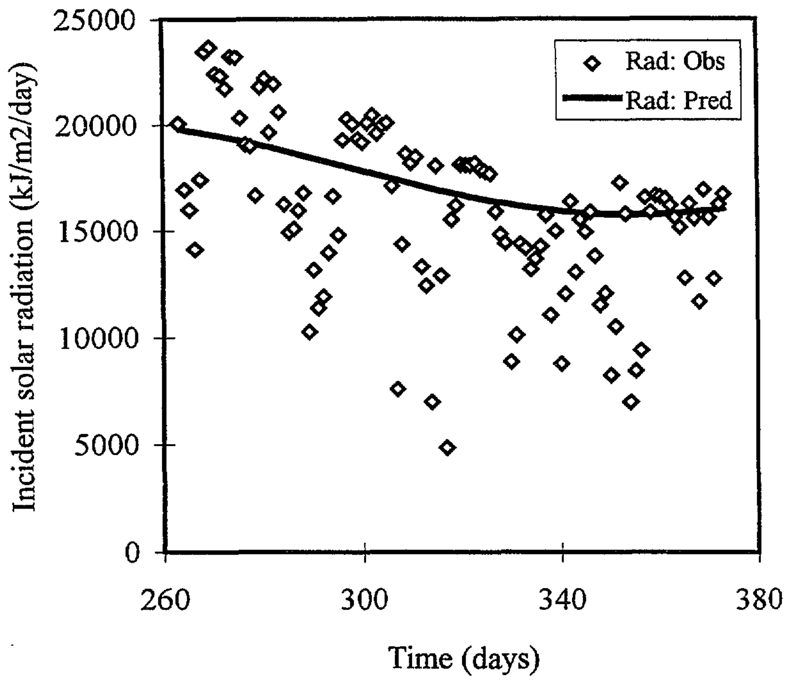

Figure 8. Incident short-wave solar radiation predicted by the use of the weather model in *POND* compared to actual data for the El Carao site. Simulation assumed scattered cloud conditions with cloud cover set to 0.5.

These results suggest that the weather generator may not provide sufficiently accurate predictions of solar radiation (Figs. 7-9) which may be related to the use of a constant cloud cover, a highly variable parameter. On the other hand, empirical equations from Straskraba and Gnauck (1985) predicted both minimum and maximum air temperatures reasonably well across the tested sites (Figs. 10-12).

The pond water temperature model has also been validated for three CRSP sites in Thailand Honduras, and Rwanda (Figs. 13-15). As expected, model accuracy is improved when actual weather data are used. In general, *POND* appears to predict water temperature with adequate accuracy across sites that are geographically distinct. Although the weather generator does provide an alternative in the absence of actual weather data, it should be used with caution, especially for solar radiation prediction when cloud cover varies substantially on a day to day basis. The solar radiation generator is also likely to provide poor predictions for atypical seasons (i.e., when radiation values differ substantially from mean values for the site; see Fig. 7). Use of more sophisticated weather generators (e.g., Richardson and Wright, 1984; Geng et al., 1988) may be an alternative that would result in better predictions of incident short-wave solar radiation. Another source of discrepancy between observed and predicted water temperatures generated by the use of the weather model may be the use of constant wind speeds in the simulation runs. A stochastic approach to the generation of wind speed (which requires specification of a user-specified mean and standard deviation as used by Neto and Piedrahita, 1995) and cloud cover data, both of which assume that these two variables are normally distributed, has been implemented in *POND*. We anticipate testing this approach as a means of improving weather predictions over the next few months.

Water temperature simulated by *POND* will likely be sufficient for most planning and long-term management applications (e.g., site assessment in relation to targeted culture species, water chemistry calculations, estimation of fertilizer and feed requirements, and economic analysis). However, for situations when it is desirable to predict the onset and extent of stratification events such as might be the case for short-term management applications (e.g., to examine whether destratification devices should be used), stratified models (e.g., Losordo and Piedrahita, 1991; Culberson and Piedrahita, 1992) are perhaps more suitable.

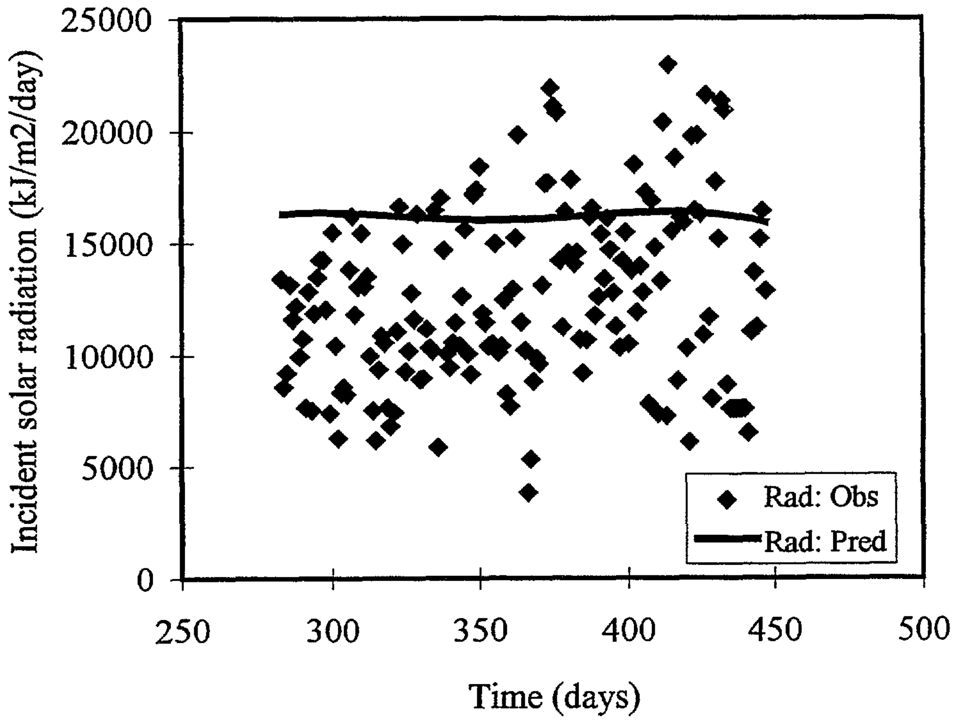

Figure 9. Incident solar radiation predicted by the use of the weather model in *POND* compared to observed data for the Rwasave, Rwanda site. Simulation assumed broken cloud conditions with cloud cover set to 0.75.

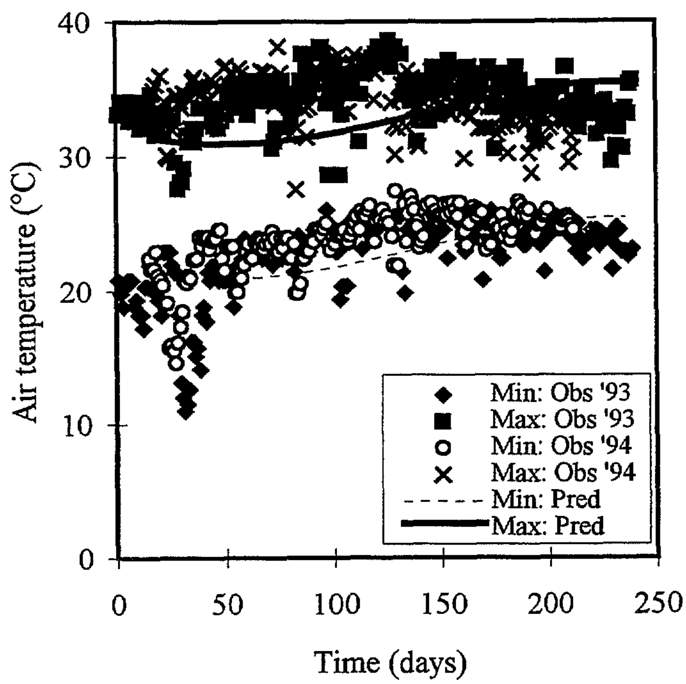

Figure 10. Minimum and maximum air temperatures predicted by the use of weather model in *POND* compared to observed data (recorded in 1993 and 1994) for the Bang Sai site. Daily temperature amplitude was assumed to be 10°C.

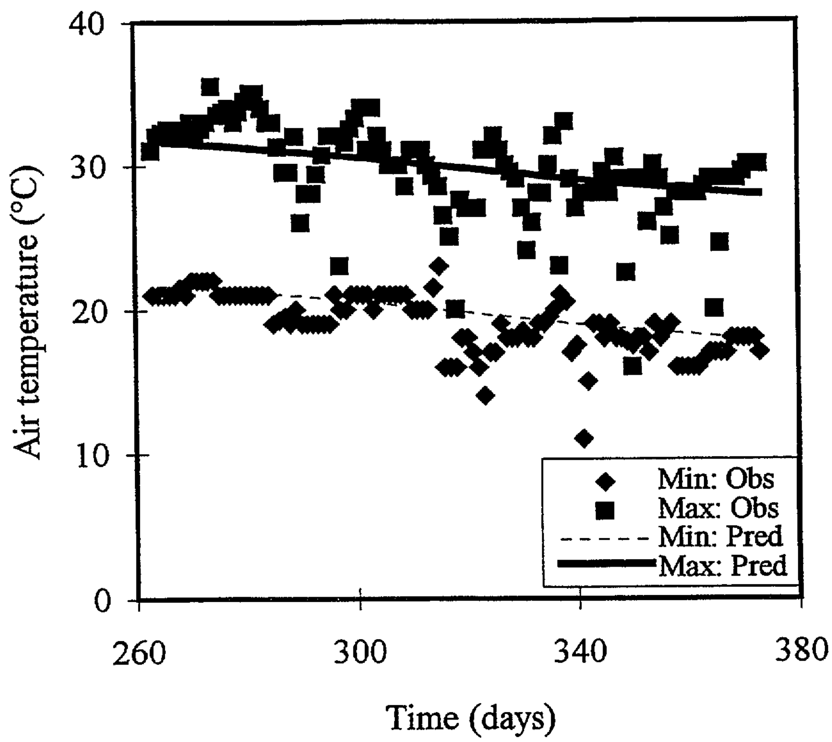

Figure 11. Minimum and maximum air temperatures predicted by the use of the weather model in *POND* compared to actual data for the El Carao site. Daily air temperature amplitude was assumed to be 10°C.

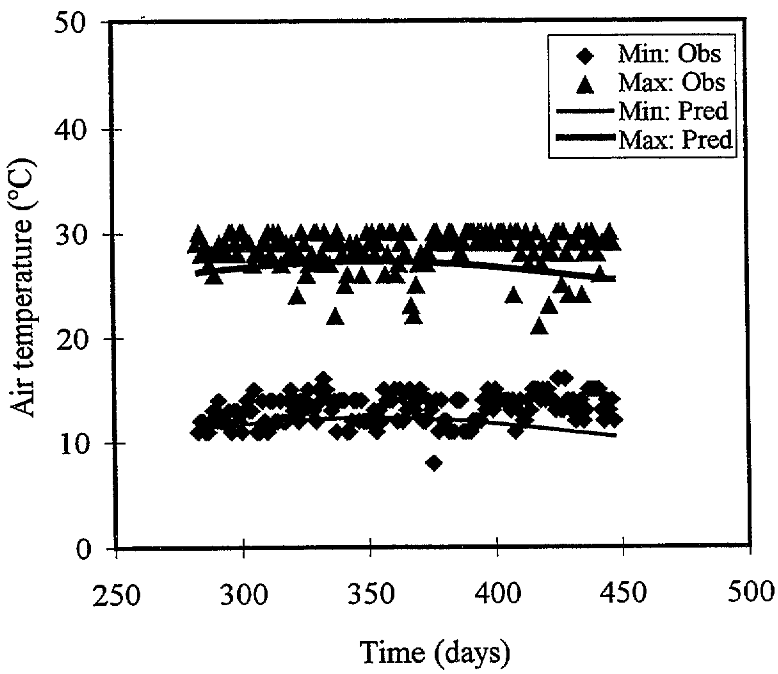

Figure 12. Minimum and maximum air temperatures predicted by the use of the weather model in *POND* compared to observed data (recorded in 1989-90) for the Rwasave, Rwanda site. Daily temperature amplitude was assumed to be 15°C.

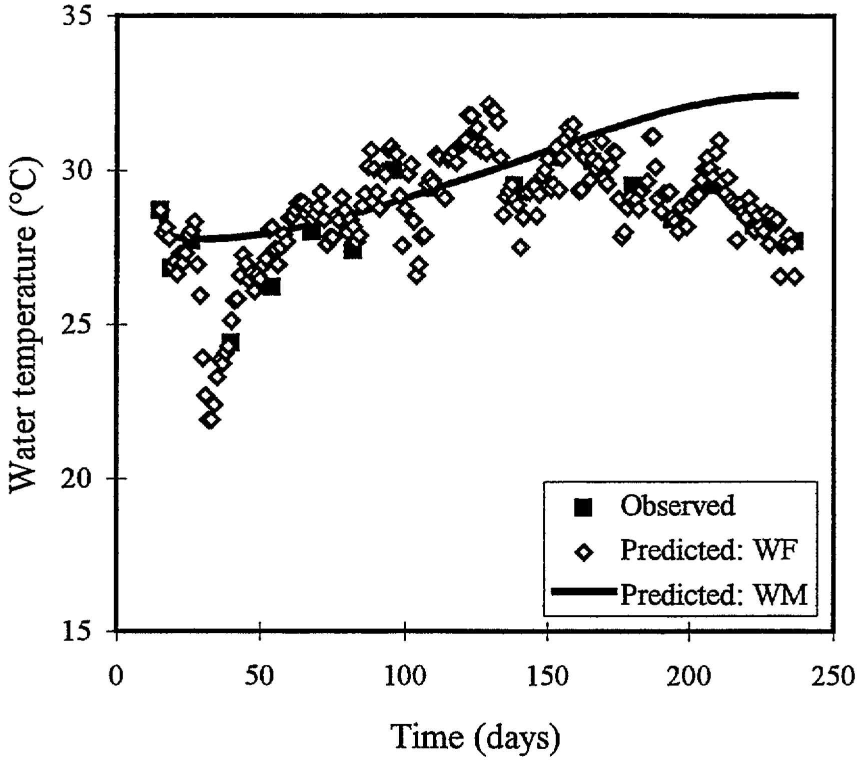

Figure 13. Pond water temperatures predicted by *POND* for the Bang Sai site. Weather data were generated by the model in *POND* (WM) or read from ASCII files (WF). A constant relative humidity of 80% was assumed.

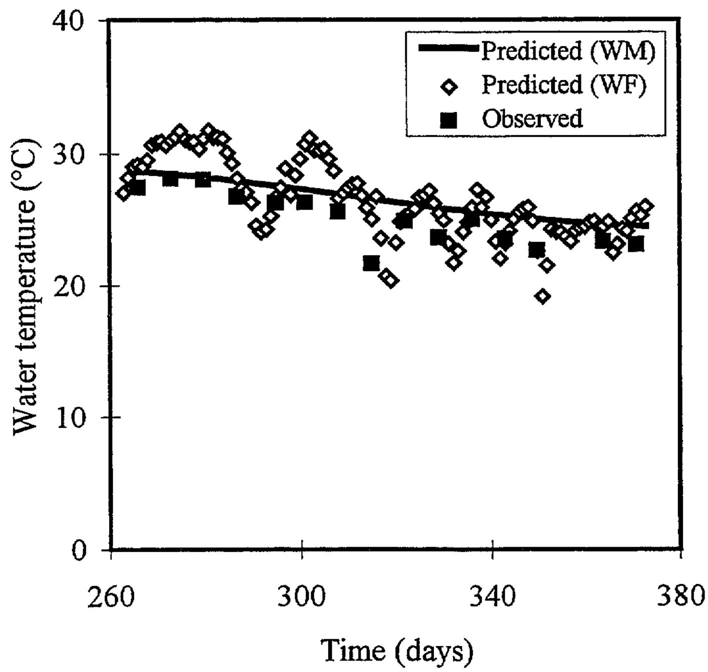

Figure 14. Pond water temperature predicted by *POND* for the El Carao site. Weather data were generated by the model in *POND* (WM) or read from ASCII files (WF). A constant relative humidity of 80% was assumed.

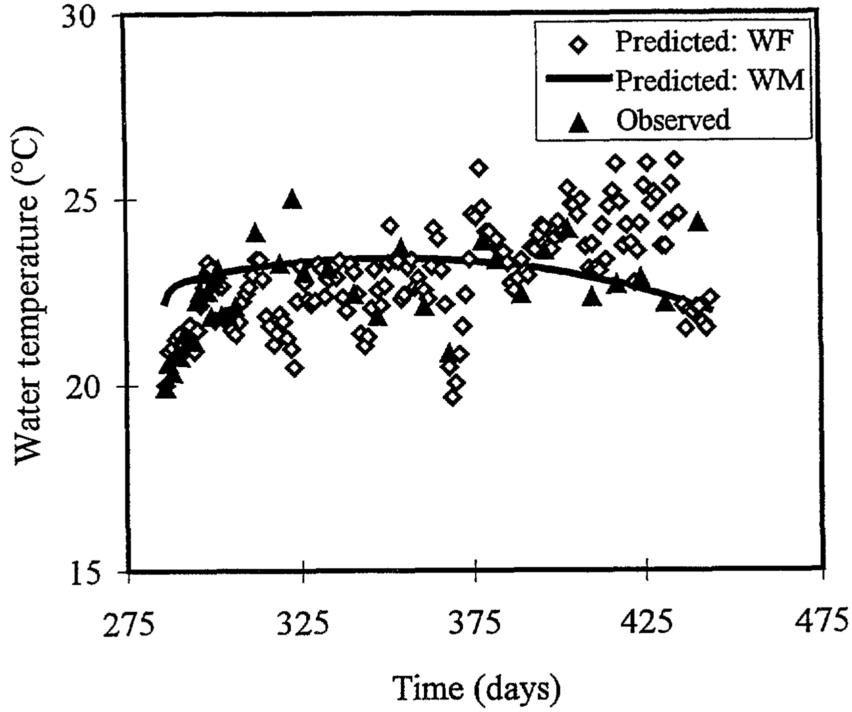

Figure 15. Pond water temperature predicted by *POND* for the Rwasave, Rwanda site. Weather data were generated by the model in *POND* (WM) or read form ASCII files (WF). A constant relative humidity of 80% was assumed.

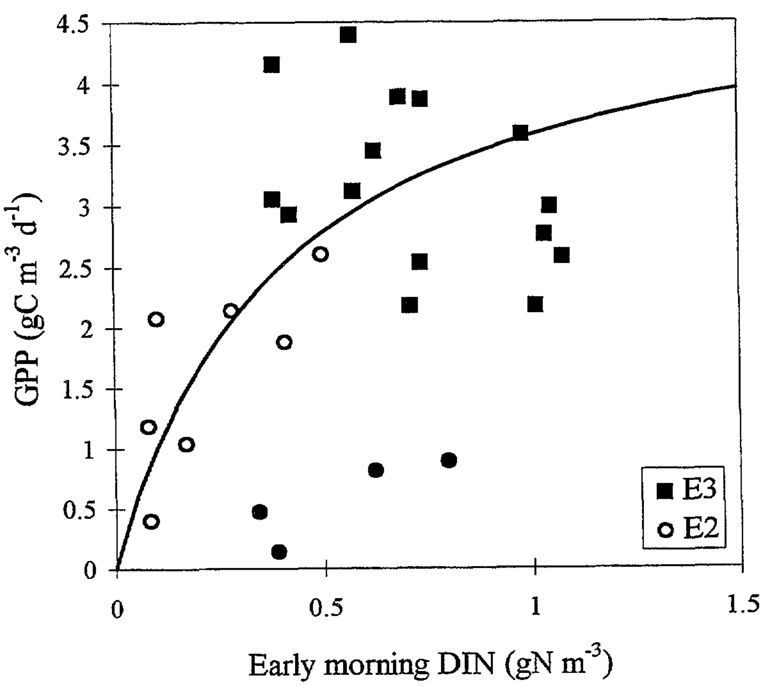

Figure 16. Gross primary productivity (GPP) in relation to dissolved inorganic nitrogen (DIN) concentrations in two ponds at Bang Sai. Dark circles represent the E2 ponds that were severely phosphorous limited (see Figure 17).

Nutrients

As indicated earlier, the Level 1 models in *POND* can be used to estimate fertilizer requirements based on a modified approach of the *PONDCLASS* fertilization method. Preliminary results for one of the CRSP sites (Bang Sai) are presented here. Two GPP rates of 4 and 6 g C m^{-3} d^{-1} were assumed for this site. The effect of a higher GPP rate is to increase fertilizer requirements for a pond. CRSP data for this site suggest that inorganic nitrogen and phosphorus concentrations of 0.5 and 0.2 g m^{-3} may be required in ponds to ensure that these two factors do not limit phytoplankton growth (Fig. 16-17). Based on these assumptions, daily nutrient requirements for a pond at this location as predicted by the *POND* Level 1 models are indicated in Fig. 18. The sudden decrease in fertilizer requirements around day 35 coincided with low water temperature, which severely limited productivity rate as predicted by the model. Estimated mean requirements for the higher GPP rate roughly correspond to nitrogen and phosphorus fertilization rates of 25.5 and 4.2 kg ha^{-1} wk^{-1} respectively, both of which are within the range that CRSP researchers have found to be appropriate for this site. The simulations assume that 50% of the nitrogen and phosphorus taken up by algae are recycled via respiration and death, and that there is a 25% loss of these nutrients to fish biomass (which accounts for the increase in nutrient requirements towards the end of the run). These parameter values are fairly consistent with pond nutrient budget studies (e.g., Schroeder, 1987; see also Delince, 1992).

Further validation of the *POND* models, particularly those used at Level 2 and 3 are planned for the near future. It is anticipated that these more complex modelling approaches will continue to be useful tools to understand pond dynamics processes and help in the formulation of more practical approaches of predicting resource requirements for efficient pond management that will ultimately be implemented in the Level 1 framework.

Applications of POND

Decision support systems such as *POND* can provide valuable information in the context of pond management, planning, extension (including technology transfer) and research. Specific applications where *POND* is likely to be useful within each of these broad focus areas are discussed in greater detail below (see also Fig. 1).

Species/Facility Customization

The *POND* framework is generic in that it can be adapted for different species and culture conditions. This feature will be useful for pond managers who wish to explore the use of alternate species or want to compare model output and recommendations (e.g., feeding or fertilization rates) to their current practices. Such analyses may also be important for planning, research and extension activities (e.g., feasibility studies for different species and/or locations). To demonstrate the use of *POND* for such analyses, simulations were conducted for El Carao site in Honduras to compare Nile tilapia and tambaquí growth and yields. The grow-out period was assumed to be 150 d; tilapia were stocked at 1 fish m^{-2} in fertilized and fed ponds, and tambaquí stocked at 3 fish m^{-2} in fed ponds. A final yield of 2387 kg ha^{-1} (mean fish weight of 266g) was predicted by the *POND* model for the grow-out period. Total feed, nitrogen and phosphorus requirements amounted to about 1566 kg ha^{-1}, 400 kg ha^{-1} (2.7 kg ha^{-1} d^{-1}) and 79 kg ha^{-1} (0.5 kg ha^{-1} d^{-1}). For tambaquí, the yields were 2524 kg ha^{-1} (mean fish weight of 126g), and feed requirements 5324 kg ha^{-1}. These results are relatively consistent with experimental studies (Green et al., 1994; Teichert-Coddington, in review). Factoring in local costs for various resources by the use of the *POND* enterprise budgets and market demands will assist pond planners and managers in selecting the appropriate species and culture techniques for their location.

Economic Optimization

The simulation and economic analysis capabilities of *POND* can be useful for economic optimization. From a management perspective, such analyses may focus on identifying suitable practices (e.g., levels of fertilization and feeding, water exchange) for an already existing facility. From a planning perspective, optimization may provide useful information for feasibility studies that focus both on facility configurations (e.g., combinations of ponds, lots and species) and management strategies. Optimization may also be of interest to researchers involved in the comparison of economic benefits from different pond aquaculture systems. For example, test simulations were conducted in fertilized and fed ponds in El Carao to compare economic benefits of different culture systems for Nile tilapia. Yields for 150 d runs were as follows: 1140 kg ha^{-1} in fertilized-only ponds (stocking density of 1 fish m^{-2}), 2463 kg ha^{-1} in fed ponds (constant feeding rate at 3% of the

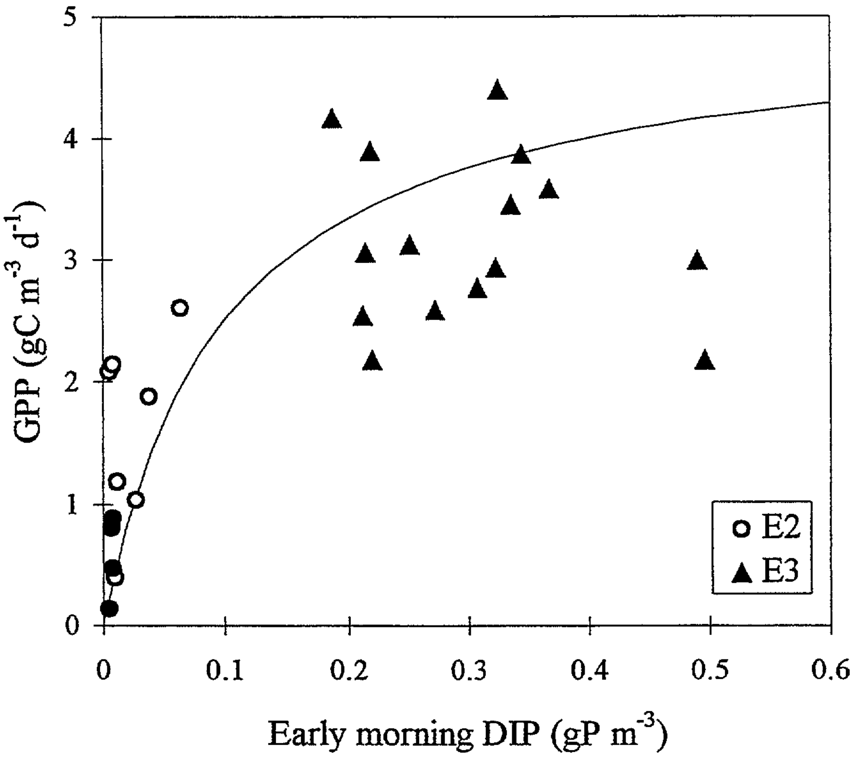

Figure 17. Gross primary productivity (GPP) in relation to dissolved inorganic phosphorous (DIP) concentrations in two ponds at Bang Sai. Dark circles represent the E2 ponds that had a surplus of nitrogen (see Figure 16).

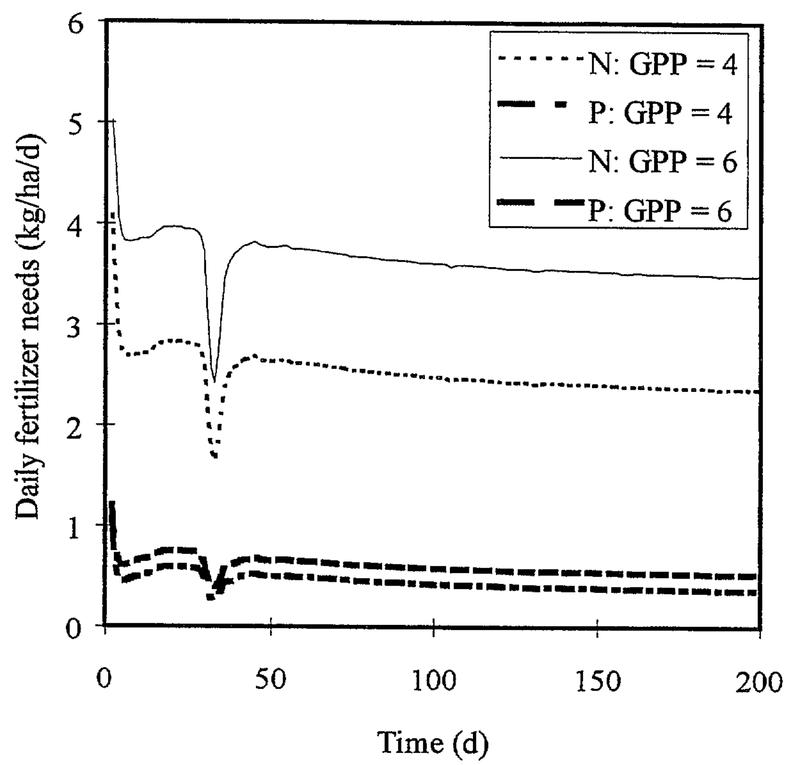

Figure 18. Daily nitrogen (N) and phosphorous (P) requirements predicted by the Level 1 model at two rates of maximum, light-limited GPP (4 and 6 g C $m^{-3}d^{-1}$).

body weight and stocking density of 1 fish m^{-2}), 2188 kg ha^{-1} in fed ponds (fish fed at 1.5% of the body weight after the first month of fertilization and then at 3% for the last 1.5 months; stocking density of 1 fish m^{-2}) and 4111 kg ha in fed ponds (constant feeding rate at 3% of the body weight and stocking density of 2 fish m^{-2}). Corresponding economic returns (using cost data from Green et al., 1994) were: -1542, 8786, 8123 and 21955 Lempiras respectively. At these local costs, it would appear that the final treatment is optimal. However, it is possible that the higher stocking density and feeding rate may cause water quality problems (which may in turn increase the costs of water quality management and risks associated with this culture system) that are not accounted for in the simulations.

Lot Management

POND managers may be interested in exploring the effects of stocking single or multiple fish species at different densities in ponds. It may also be of interest to examine the effects of stocking and harvesting lots at different dates on production and facility-level economics. For example, Level 1 simulations were conducted to examine the effects of stocking dates on Nile tilapia growth stocked at 2 fish m^{-2} in fertilized and fed ponds at El Carao, Honduras. Stocking dates for the three ponds simulated were January 1, April 15, and July 15 respectively and corresponding harvest dates June 15, September 30, and December 31. Predicted fish weights were 215, 286 and 203 g respectively for the ponds stocked on January 1, April 15, and July 15. These differences in fish weight, which are likely to affect production economics, were apparently caused by more favorable temperatures during the period April-September. Such types of analyses may also be useful in the context of scheduling pond operations and assessing resource needs (e.g., fingerling requirements). Extension agents may also find it beneficial to use *POND* as a tool in recommending appropriate stocking densities to farmers based on local conditions.

Estimation of Feed Requirements

Artificial feeds often represent the single most important component of variable costs in an aquaculture facility. This is especially true of large-scale commercial operations. Therefore, assessment of feed requirements (in terms of both quantity and quality) and subsequent effects on facility-level economics (and water/sediment quality) will likely be useful for a wide variety of personnel involved in pond aquaculture. As an example, Level 1 simulations of catfish production for a 150 d period at Stoneville, Mississippi with feeding rates set to 60, 80 and 100% satiation results in final fish weights of 200, 310, and 455 g respectively. Feed requirements were 6500, 10440, and 15470 kg ha^{-1} for the low, medium and high feeding intensity treatments. Such estimates are required for large aquaculture operators (regardless of the target species) so as to manage feed inventory properly and gauge the economic benefits of different feeding rates.

Estimation of Fertilizer and Lime Requirements

In *POND*, fertilizer requirements can either be generated by the use of guidelines proposed by Lannan (1993), specified by the user or generated by the models. Each of these options serve different needs. Thus, the fertilization guidelines of Lannan (1993) allow pond managers to adjust fertilization rates on a routine basis. This is beneficial from the viewpoint of reducing fertilizer waste (if any) and provides for interactive pond management. Generation of fertilization schedules (Fig. 18) as one of the outputs of the *POND* models is useful from the viewpoint of assessing fertilizer requirements for an entire facility and gauging the viability of certain pond aquaculture systems (e.g., subsistence farming) from a planning perspective.

Finally, the use of fertilizer application rates defined by pond managers as input data to the *POND* models will provide opportunity in the future to compare model outputs (e.g., fish growth, plankton growth, and/or nutrient concentrations) with actual pond information. Such analyses will likely provide opportunity for future model refinement.

Lime requirements recommended by *POND* are likely to be more applicable to newer ponds without extensive organic matter deposits (J.R. Bowman, Oregon State University, personal comm.., 1995). Older ponds may have different lime requirements because the original nature of the soil is modified as its organic matter content accumulates.

Water and Sediment Quality Management

Analyses of the effects of management practices on pond water/sediment quality are important from planning, extension and management perspectives in terms of resource flows, facility-level economics and verifying whether effluent standards (e.g., nitrogen, phosphorus and organic carbon levels) are met. It may also be possible to assess short-term aeration requirements for ponds by the use of Level 3 models once they are fully validated. Because many pond processes are not fully understood, the *POND* models may also be used to guide experimental work that specifically focuses on these processes. At the current time, Level 1 and 2 models provide some capability in predicting and estimating nutrient sources and sinks in ponds; however, further validation work is required to expand such functionality.

Future Directions

As indicated earlier, current research on *POND* focuses on validation of different models in the software. Further, an effort is underway to combine facility-level pond and crop simulations for a thorough analysis of integrated farming systems. Our experience with the software suggests that it is likely to be a useful tool for facility-level analysis. Applications of the *POND* Level 1 models for the assessment of regional-scale pond aquaculture potential are in progress with collaborators from the FAO.

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Decision Support for Pond Aquaculture: Parameter Estimation for Simulation Models

Work Plan 7, DAST Study 5

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Introduction

Users of the *POND* decision support system may often be interested in tailoring the models to one or more fish species at a given location. Manual calibration of the fish bioenergetics model in POND for Nile tilapia *(Oreochromis niloticus)* growth has previously been reported (Bolte et al., 1995). However, estimating suitable parameters for complex, non-linear simulation models by manual calibration can be extremely tedious, because of the potentially high degree of interaction between variables and the large size and dimensions of parameter spaces to be searched.

Several traditional approaches to non-linear parameter estimation have been developed in the past. These traditional approaches typically involve definition of an objective function (e.g., error sum of squares between predicted and observed values' absolute error) that is to be minimized, and a procedure for finding a set of parameters which in fact minimize this function. Thus, model parameter estimation may be considered to be an optimization process. In general, numerical algorithms for minimizing the objective function require evaluation of partial derivatives of the objective function with respect to each of the parameters to be estimated (Fig. 1). Such derivative evaluations for large models can rarely be accomplished analytically, and are usually calculated numerically (Bard, 1974). However, it is difficult to achieve accurate results with numerical methods for derivative calculations, and the techniques that do exist tend to be relatively computation-intensive. In general, the difficulties associated with these methods have severely limited their application in biological models.

Recently, however, a new class of non-linear optimization strategies has been developed which show considerable promise for non-linear model estimation. This class includes genetic algorithms (GA's), a fairly recent optimization technique that attempts to overcome the problems of traditional methods (Schwefel, 1981; Davis, 1992). A GA may be thought of as an implementation of the process of natural selection or evolutionary adaptation on a computer (Holland, 1975), evaluating populations of solutions based on natural selection, and allowing the most fit of these solutions to combine and reproduce until highly suited individuals emerge, representing good solutions to the problem at hand. GA's have been previously used as a tool for parameter estimation of complex agricultural simulation models (Sequeira and Olson, 1995). A study was initiated to examine the use of GA's for estimating suitable bioenergetic parameters for different pond species.

Methods

Three species were chosen to examine the suitability of a GA-based parameter estimation technique for the POND models. The selected species were channel catfish *(Ictalurus punctatus)*, tambaquí *(Colossoma macropomum)* and pacu *(Piaractus mitrei)*. Growth, water temperature and feeding data were obtained from the following sources:

Channel catfish: Robinson and Li (1995)
Tambaquí: Merola and Pagan-Font (1988)
Pacu: Lima et al. (1988)

Table 1. Bioenergetic parameters for channel catfish, tambaquí, and pacu estimated by the use of GAs.

PARAMETER	CATFISH	TAMBAQUI´	PACU
Anabolism parameters			
Efficiency of assimilation	0.7865	0.6695	0.7719
Anabolism exponent	0.6327	0.6855	0.7154
Food consumption coefficient	0.2885	0.2863	0.2415
Catabolism parameters			
Feeding catabolism coefficient	0.1133	0.1057	0.0529
Catabolism exponent	0.5118	0.5336	0.5332
Minimum catabolism coefficient	0.0227	0.0146	0.0094
Temperature parameter	0.0119	0.0111	0.0290
Temperature scalers			
Minimum	13.31	14.40	17.46
Maximum	35.96	38.58	31.36
Optimum	30.81	29.04	28.09

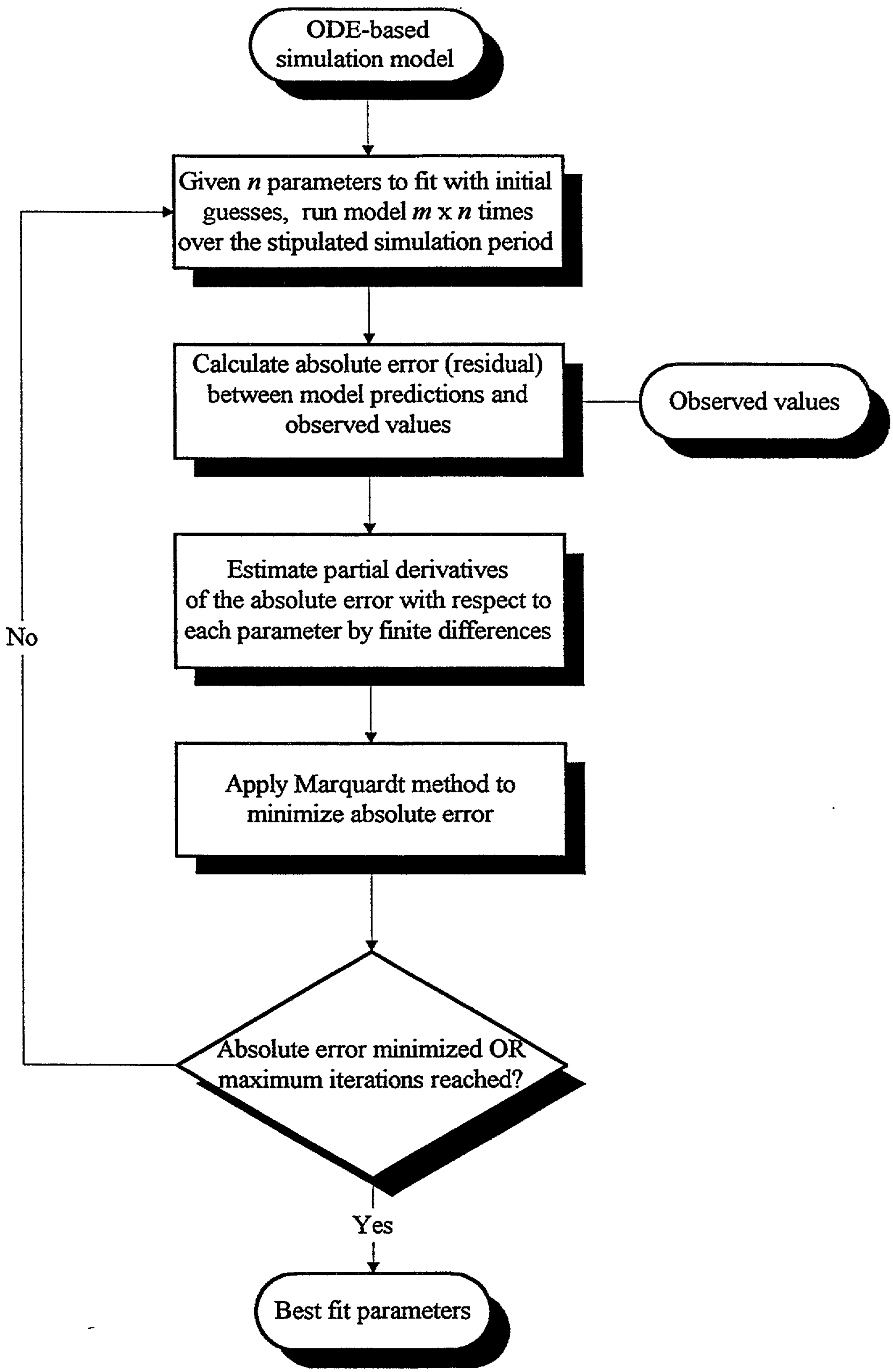

Figure 1. Summary of the procedure for parameter estimation for an ordinary differential equation (ODE) based simulation model by a non-linear regression technique (Marquardti's method).

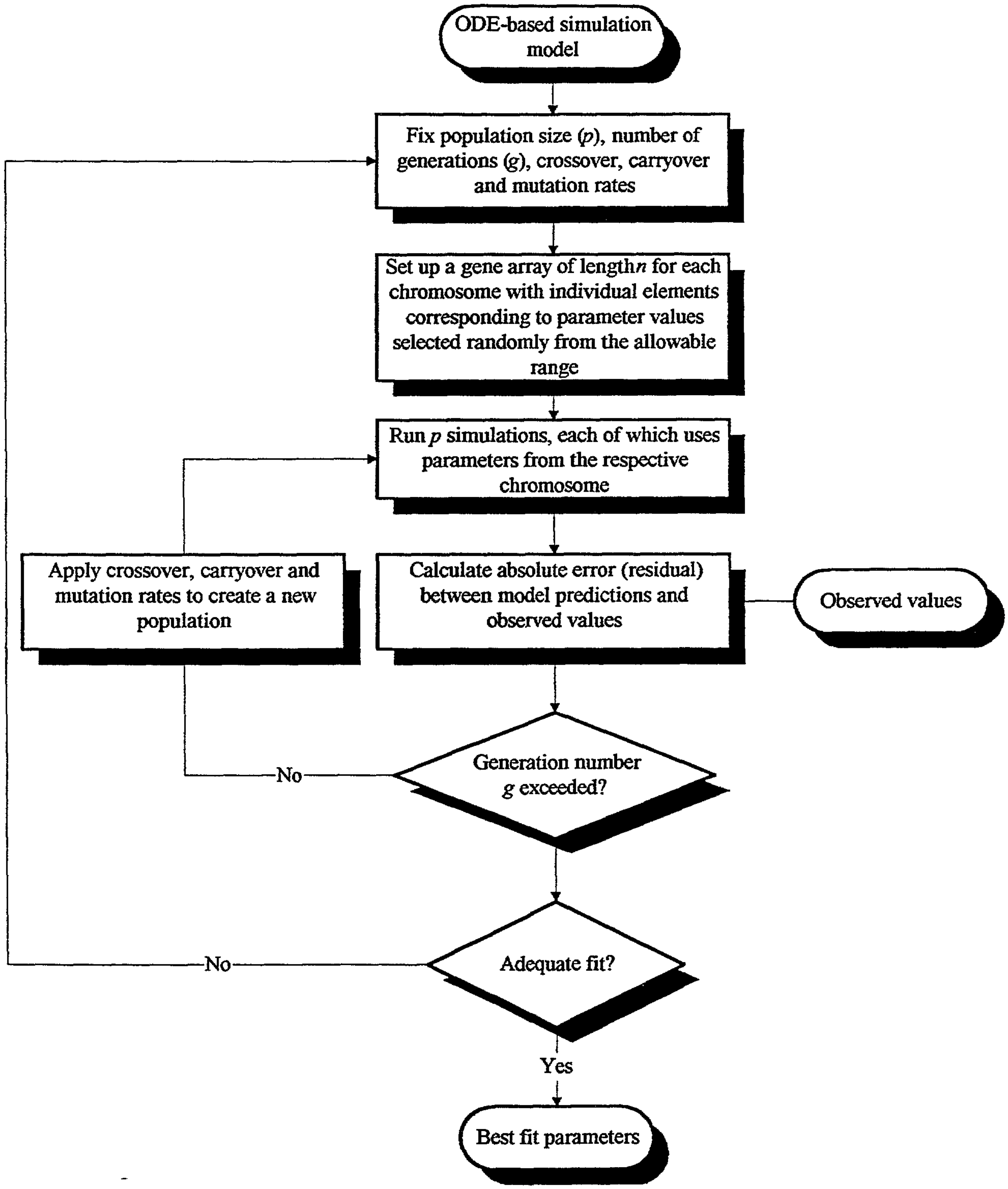

Figure 2. Summary of the procedure for parameter estimation for an ordinary differential equation (ODE) based simulation model by GAs.

The objective function chosen for optimization was the minimization of the absolute error between observed and predicted growth data. Populations of individuals were created, with each individual consisting of a number of genes. Each gene represented one value of a specific parameter; an individual then represented a collection of parameter values corresponding to one complete parameter specification. The population represented a range of potential parameter values and combinations.

The search for an optimal set of parameters was initiated by running the POND model for each individual in the initial (random) population, and comparing the predicted results of the model for that individual's parameter set with observed results. Fitness of that individual was determined by the absolute error between observed and predicted results. After evaluating all individuals in the population, the most fit individuals were selected for crossover and mutation, resulting in a new, more fit population. This process was repeated through multiple generations, until an individual with adequate fitness was found. This individual contained a parameter set that provide good fit with between the model and observed results. The approach used for parameter estimation is summarized in Fig. 2.

Results

Adequate convergence was typically for all species using a generation size of 20 with carryover, crossover and mutation rates set to 0.2, 0.25 and 0.15 respectively. Model fits are indicated in Fig. 3 and estimated parameters in Table 1. In general, the genetic algorithm was able to find a good combination of parameters after 5 to 10 generations.

Discussion

Predicted fish growth profiles for these species are compared to observed data in Fig. 3. For these simulations, water temperature values as reported in the data sources were used. Simulated fish weights for catfish are very similar to observed data.

The predicted weight for tambaquí during the initial culture period is also very similar to observed data (Fig. 3); however, weight during the exponential growth phase of this species showed some deviation from observed values, although the final weights are very comparable to observed data. Steady state growth rates during the final two months of culture

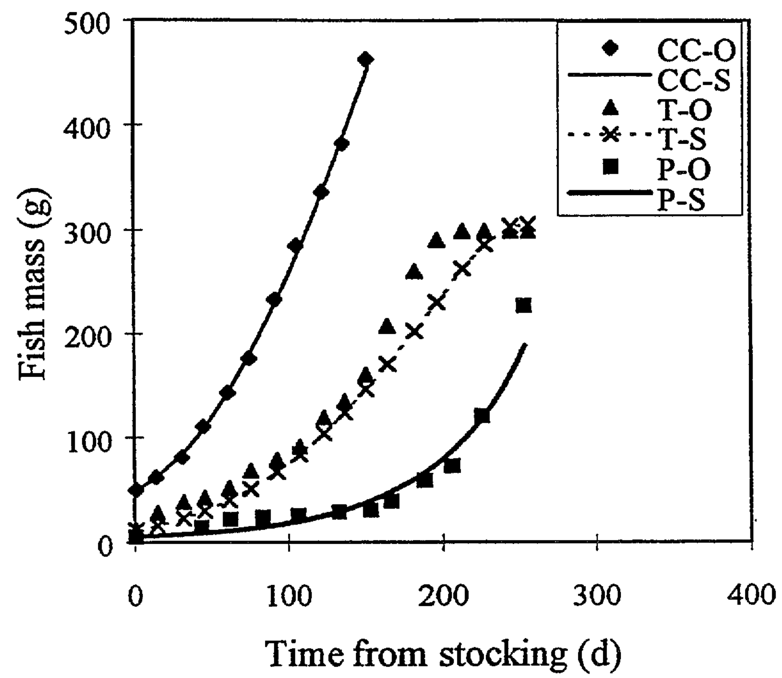

Figure 3. Simulated (S) and observed (O) fish growth profiles for channel catfish, tambaquí, and pacu. All the simulated profiles generated by the use of the POND models with parameters estimated by GAs.

were apparently caused by unseasonably low water temperatures (Merola and Pagan-Font, 1988). Predicted fish weights for pacu are comparable to observed data, except for the last data point when this species appeared to be entering into a very rapid growth phase.

Model calibration results suggest that the energetics model is likely to provide adequate accuracy for most applications (e.g., seasonal yield projections). However, it is possible that one set of model parameters may not be suitable for all growth phases, especially for species (e.g., tambaquí and pacu) which enter into a very rapid growth phase when temperatures are optimal. In such situations or when more accurate predictions are desirable, it may be advisable to separately parameterize the model for the growth of fingerlings and food fish.

Running on personal computers, genetic algorithms proved to be an acceptable approach for the complex task of parameter estimation for the nonlinear models upon which POND is constructed. Because the approach proved quite successful at producing site-specific and species/variety-specific growth parameter estimated, it has been incorporated directly into the POND software.

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Central Data Base

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Summary of Activities

At the time the Central Data Base was transferred to the University of Hawaii it was complete through the Fourth Work Plan and nearly complete through the Fifth Work Plan. Since then data from two experiments in Rwanda and two experiments in Thailand were added to the Fifth Work Plan data.

The Sixth Work Plan included 19 experiments to be conducted between September 1, 1991 and August 31, 1993. Supplemental Work Plans included nine more experiments to be conducted during that period. All data collected using standard CRSP methods, and which the database could accommodate, were to be reported. (See Sixth Work Plan, page 1). Currently, the CRSP data base includes the following Sixth Work Plan data: two experiments from Honduras, three experiments from the Philippines and five experiments from Thailand. There are no data from Rwanda reported in the data base. In summary, ten experiments from the Sixth Work Plan have been included in the data base.

The Seventh Work Plan experiments covered the period from September 1, 1993 to August 31, 1995. Nine experiments were to be conducted in Honduras; none are reported in the database. Ten studies were to be conducted in Thailand; the results of four of those studies are included in the data base. Two experiments from the Philippines are reported. No data from Egypt are included in the database. Rwanda data are understandably absent. In summary, six experiments from the Seventh Work Plan have been added to the database.

Central America

Researchers at the Choluteca station characterized shrimp farm effluents as the first step in estimating the carrying capacities of local estuarine systems for shrimp. Intake and discharge from shrimp farms located on the estuaries of the Gulf of Fonseca were sampled during both the rainy and dry seasons in 1993-94. Results showed a mean net consumption of inorganic nitrogen and phosphorus and a mean net discharge of organic matter. Most of the nitrogen entered and left the ponds through water exchange; most phosphorus entered the ponds as feed but left by water exchange. Pond discharge of both nitrogen and phosphorus increased linearly with the feed conversion ratio. The conversion of feed and nitrogen to shrimp flesh was greater during the wet season than the dry season.

Taura Syndrome is the cause of high mortality in some Central American shrimp ponds. In response to an urgent need for information on how to manage ponds affected by Taura Syndrome, researchers at the Choluteca station investigated the relationships among stocking density, survival, and shrimp yield in affected ponds. *Penaeus vannamei* were stocked in ponds on two farms during the wet season and on three farms during the dry season. At each farm, four different stocking rates were used. Researchers found no significant correlation between stocking density and survival during either the wet or dry season, nor did they find a seasonal influence on survival. Shrimp production rose with increased density, regardless of the season. Farmers' net income increased with density during the wet season, but decreased or remained neutral with an increase in density in the dry season, because income is related to both biomass and shrimp size. During the wet season, production increased without a decrease in size of harvested shrimp; however, during the dry season, mean shrimp size decreased.

In addition, researchers stationed in Central America conducted activities that were originally scheduled for the Rwanda site (see East Africa).

Characterization of Shrimp Farm Effluents in Honduras and Chemical Budget of Selected Nutrients

Work Plan 7, Honduras Study 2

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Introduction

Chemical budgets of aquacultural facilities are useful for water quality management and quantification of effluent impact on receiving waters. Characterization of farm effluents is prerequisite to estimating the carrying capacities of local estuarine systems for shrimp culture.

The percentage of world shrimp production in Central America is only about 7% (Rosenberry, 1994), but the industry is important to local economies. In Honduras, over $70 million was generated by shrimp culture in 1994 placing the industry third in national export earnings. Sustainability of the industry is economically critical, and could depend on estuarine water quality. A 2-yr baseline of water quality has been established for the major shrimp producing estuaries (Teichert-Coddington, 1995). The objective of this study was to characterize farm effluents and formulate a budget for nitrogen and phosphorus.

Materials and Methods

Characterization of Water

Six farms peripherally located to the Gulf of Fonseca in southern Honduras were sampled during 1993 to 1994. Farms occupied estuaries dominated by rivers (riverine) or the Gulf (gulf embayments) (Teichert-Coddington, 1995). Intake and effluent water samples were collected from ponds every two weeks during a production cycle. Ponds were first drained and then refilled. Production cycles were sampled during dry and wet seasons, which have distinctive yield differences (Teichert-Coddington et al., 1994; Teichert-Coddington and Rodriguez, 1995). The number of ponds sampled and the seasons comprised by the sampling varied by farm (Table 1).

Water was analyzed for total settleable solids (American Public Health Association (APHA) et al., 1992), nitrate nitrogen by cadmium column reduction to nitrite (Parsons et al., 1992), nitrite nitrogen (Parsons et al. 1992), total ammonia nitrogen (Parsons et al. 1992), filterable reactive phosphate (Grasshoff et al., 1983), chlorophyll *a* (Parsons et al., 1992), total alkalinity by titration to 4.5 pH endpoint, salinity, and 2-d and 7-d BOD. Total nitrogen and total phosphorus were determined by nitrate and phosphate analysis, respectively, after simultaneous persulfate oxidation (Grasshoff et al., 1983). Organic phosphorus was calculated from the difference of total phosphorus and filterable reactive phosphate. Dissolved inorganic nitrogen (DIN) was the sum of nitrate, nitrite and total ammonia nitrogen (TAN), and organic nitrogen was the difference of total nitrogen and DIN.

Nutrients discharged during draining were determined from water samples collected at 100%, 50%, 25%, 12.5% and 0% of pond volume during a draining event.

Table 1. Characteristics of farms sampled for study.

Farm	Location	No. of ponds	Mean area (ha)	Mean duration (d)	Mean density (No./m^2)	Mean water exchange (%/d)
A	Riverine	9	27	97	6.9	5.4
B	Embayment	2	8	95	10.1	4.3
C	Riverine	2	24	104	7.3	2.6
D	Embayment	1	8.5	162	25	3.7
E	Embayment	3	6	114	9.6	8.5
F	Riverine	4	22	114	9.1	3.5
	Mean	-	20.6	106	8.9	4.8

Chemical Budgets

Inputs and outputs of total nitrogen and total phosphorus were quantified for ponds that had complete record sets. Records of feed, fertilizer, water exchange, stocking rates, and harvest weights were obtained from the producer. Concentrations of P and N were determined by analysis of intake, effluent and drainage water in each pond. Evap-oration and rainfall were not taken into account, but their effects on the nutrient budgets were probably minimal (Briggs and Funge-Smith, 1994). The general balance equation is:

$$S_{in} + F_{in} + Fert_{in} + PV_{in} + WE_{in} = S_{out} + PV_{out} + WE_{out} \pm\uparrow\downarrow$$

where S = shrimp F = feed; Fert = fertilizer; PV = pond volume; and WE = water exchange.

Feed from various farms was analyzed for N and P composition. Manufacturer's proximal analyses of feed were used in instances where independent analyses were not available. Nitrogen and phos-phorus composition of shrimp were determined by elemental analyses (Boyd and Teichert-Coddington, 1995). Percentage of dry matter, N and P for *P. vannamei* were 25.5%, 11.2% and 1.25%, respectively.

Data Analysis

Material exchange between intake and effluent water was expressed in terms of kg/ha-100 d. Calculations were based on nutrient concentration differences between intake and effluent water, daily water exchange rate, pond area and an average pond depth of 1 m. Results were grouped by estuarine type and season. Seasonal comparisons were completed for only 3 farms from which water had been monitored during both seasons. Mean differences were declared significant by non-coincidence of 95% confidence intervals. Regression analyses were used to reveal relationships between selected variables. Data were analyzed using software by Haycock et al. (1992).

Results and Discussion

Water Characterization

Water Intake

Mean intake filterable phosphate, nitrate, nitrite, total N, total P and BOD_2 were significantly higher in riverine than in embayment estuaries (Figure 1). Salinity was significantly higher in embayment estuaries. There was no significant difference for COD, chlorophyll *a*, and total alkalinity.

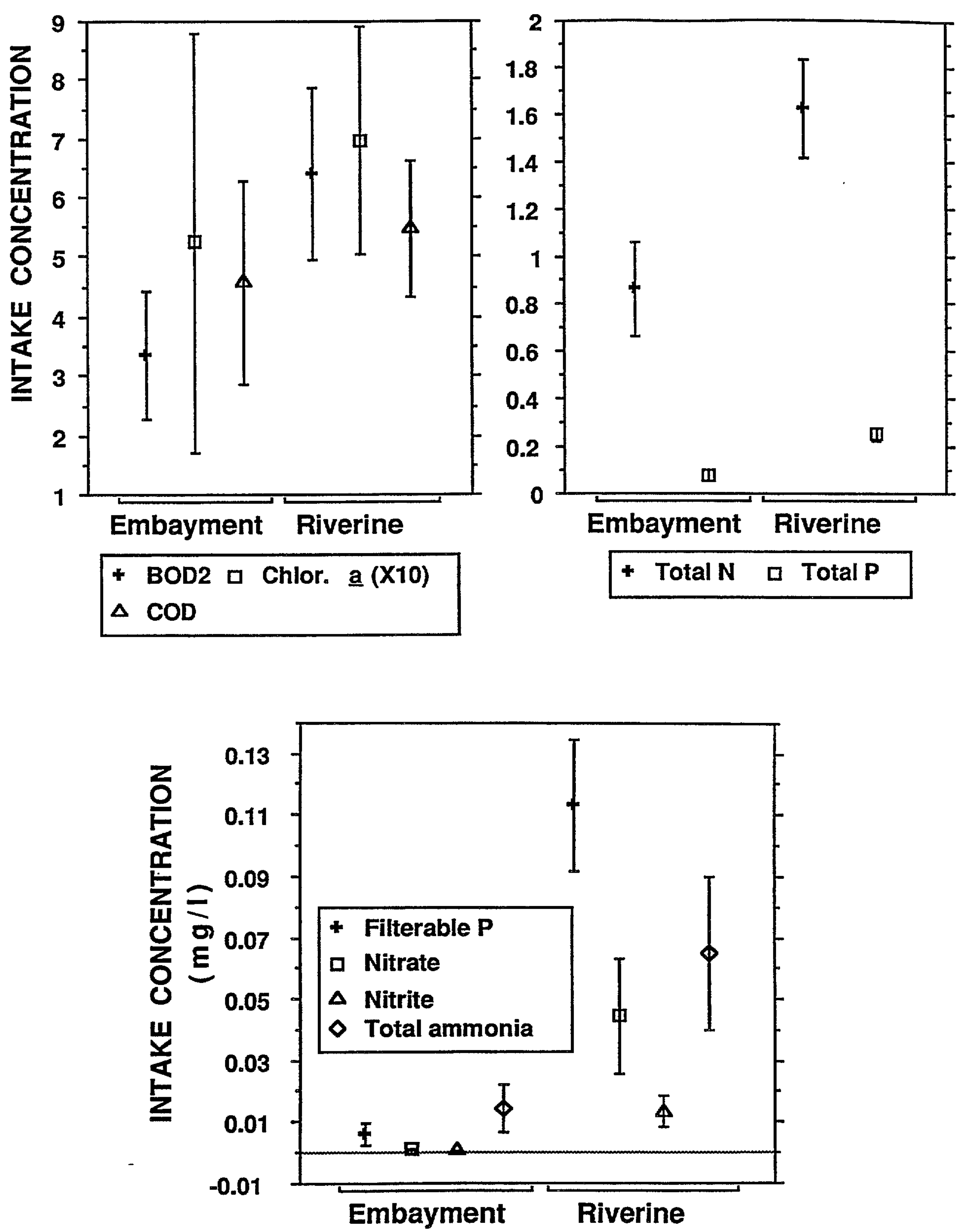


Fig 1. Characterization of farm intake water by location on Gulf of Fonseca embayments or estuaries influenced by rivers.

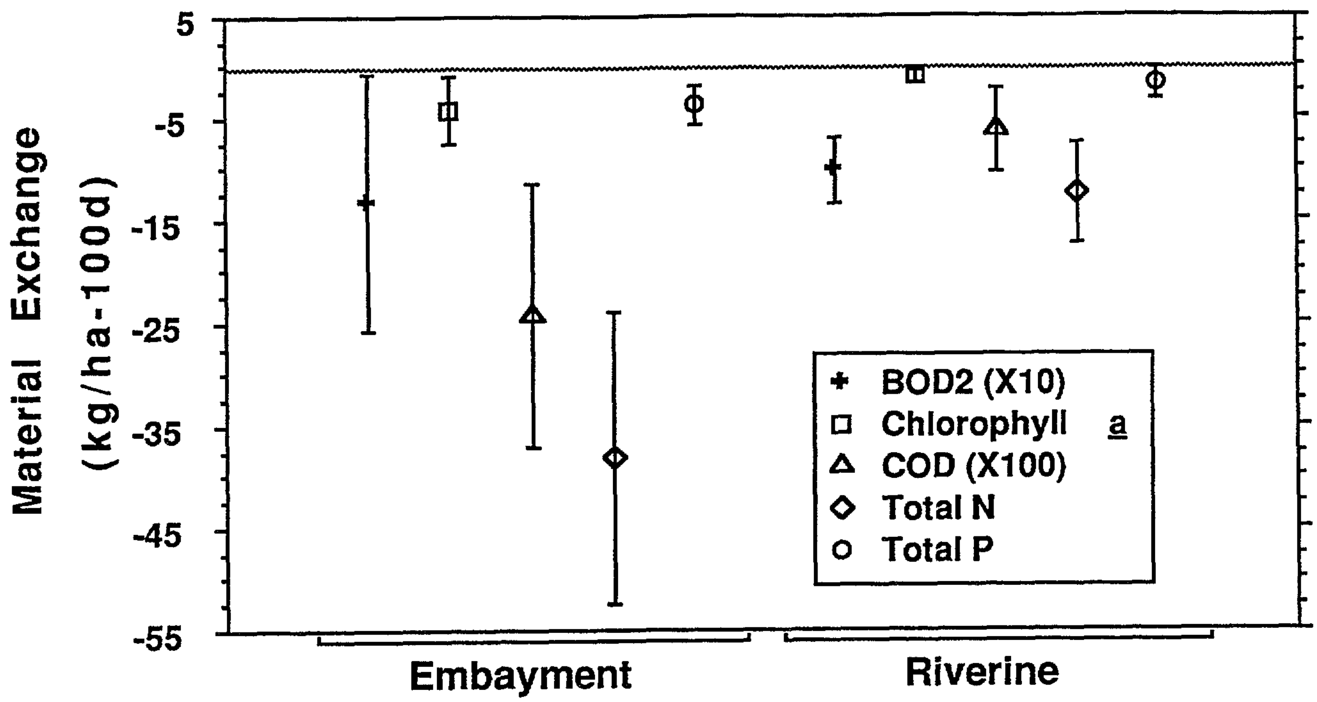


Figure 2. Mean net material (organic indicators) exchange on farms characterized by riverine or embayment location. Negative values indicate a net discharge of material.

No significant seasonal differences were found, except for total alkalinity, which was significantly higher during the dry season than the wet season.

Material exchange

Mean discharge of total N, total P, BOD_2, chlorophyll *a*, COD, total alkalinity and salinity were greater than mean intake, resulting in a net discharge of these variables from the ponds (Figure 2). On the other hand, mean discharge of nitrate, nitrite, total ammonia and filterable phosphate were less than mean intake, resulting in a net consumption of these variables (Figure 3). In summary, inorganic forms of phosphorus and nitrogen were processed into organic forms during passage through the pond.

There were some differences in material exchange depending on estuarine type. Net discharge of total nitrogen, total phosphorus, COD and chlorophyll *a* was significantly greater in embayment than riverine estuaries (Figure 2). Net consumption of total ammonia, nitrate and nitrite was significantly greater in riverine than embayment estuaries (Figure 3). No significant differences were detected for the remaining variables. Some of the variability among farms for material exchange of total ammonia and filterable phosphate stemmed from the use of inorganic fertilizers on some farms, and not on others. Where fertilizers were used, filterable phosphorus and forms of inorganic nitrogen were greater in discharge than in intake water (Table 2). There were no significant differences for material exchange attributable to season (Figure 4).

Total nitrogen and total phosphorus discharge from the Choluteca River was estimated for 1994 (Teichert-Coddington, 1995). In comparison with discharge from 11,500 ha of shrimp ponds, the river discharged 1.8 and 4.8 times more nitrogen and phosphorus, respectively (Table 3).

Table 2. Influence of inorganic fertilization on effluent concentrations of dissolved inorganic nitrogen (DIN) and filterable orthophosphate from two farms located in close proximity on the same riverine estuary. Number of ponds sampled in Farm A and B were 6 and 4, respectively.

Variable	Farm	Intake (mg/l)	Effluent (mg/l)	Difference (kg/ha-100d)	Fertili-zation
DIN	A	0.201	0.026	9.63	No
(mg/l)	B	0.100	0.037	2.21**	Yes
Filterable P	A	0.120	0.074	2.52	No
(mg/l)	B	0.139	0.226	-3.05**	Yes

** Difference was significant ($P \leq 0.01$).

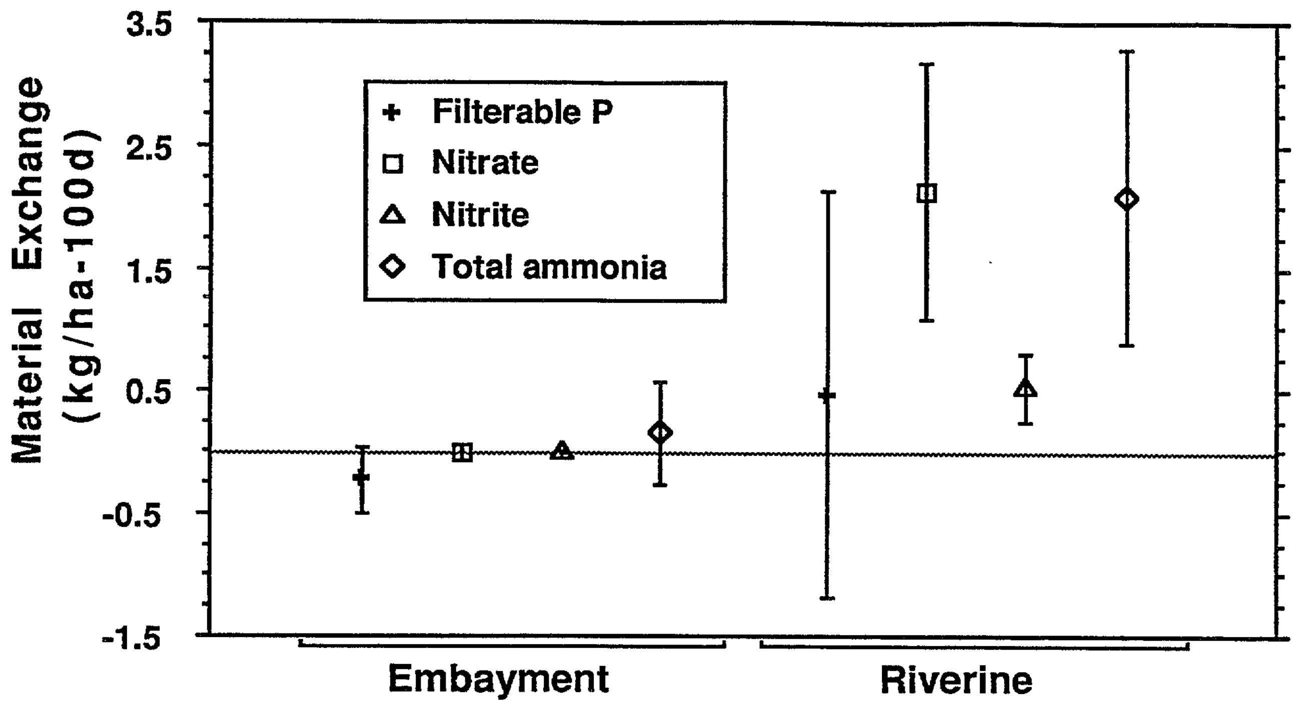


Figure 3. Mean net exchange of inorganic materials on farms characterized by riverine or embayment location. Positive values indicate a net consumption of material.

Pond draining

There was a significant inverse relationship between pond volume at drainage and concentrations of total P, total N, dissolved inorganic nitrogen, filterable phosphate and settleable solids ($P < 0.05$). Concentrations of these variables increased as pond volume decreased. However, correlation coefficients were low for these relationships, because differences during draining occurred mostly during the last 12% of water discharge (Figure 5). Suspended solids were particularly high at the end of drainage (mean = 12 ml/l) compared with the start of drainage (mean = 0.16). Despite an increase in many nutrients and settleable solids with a decrease in pond volume, mean BOD_2 remained constant from start to finish during pond drainage. There was high variation of nutrient concentration in pond drainage among farms. This variation was probably related to water control during draining; i.e., water input towards the end of drainage to provide oxygenated water to shrimp.

Nutrient Budgets

Nitrogen

Most nitrogen entered ponds with the water (58%), while feed (40%) and fertilizer (2%) accounted for the remainder of nitrogen input (Figure 6). Input by shrimp was minimal, averaging less than 0.5%.

The majority of nitrogen was discharged from the ponds with daily water exchange (72%) and pond drainage (10%) (Figure 6). Harvested shrimp accounted for 16% of nitrogen removal. Nitrogen input averaged 1% higher than the sum of nitrogen in water discharge and shrimp removal. Unobserved nitrogen was apparently fixed by soils or lost through denitrification. There was high variation among ponds, even on the same farm, for unrecorded nitrogen. It is probable that inaccurately calculated water exchange rates accounted for some of this variation.

Mean farm conversion ratios of feed nitrogen to shrimp flesh ranged from 1.4 to 4.1. From 29 to 66% of nitrogen added in the feed was not utilized by the shrimp. Nitrogen conversion ratios were directly correlated with feed conversion ratios. Nitrogen discharge from ponds, i.e., negative net material exchange, consequently increased linearly with increasing feed conversion ratios (Figure 7). The nitrogen conversion ratio was also correlated with percentage of nitrogen in the feed (Figure 8); i.e., nitrogen conversion was less efficient with increasing protein content of feed. Higher protein levels in shrimp feeds did not result in better feed conversion efficiency (Figure 9). No significant differences for feed or nitrogen conversion ratios were detected between types of estuaries.

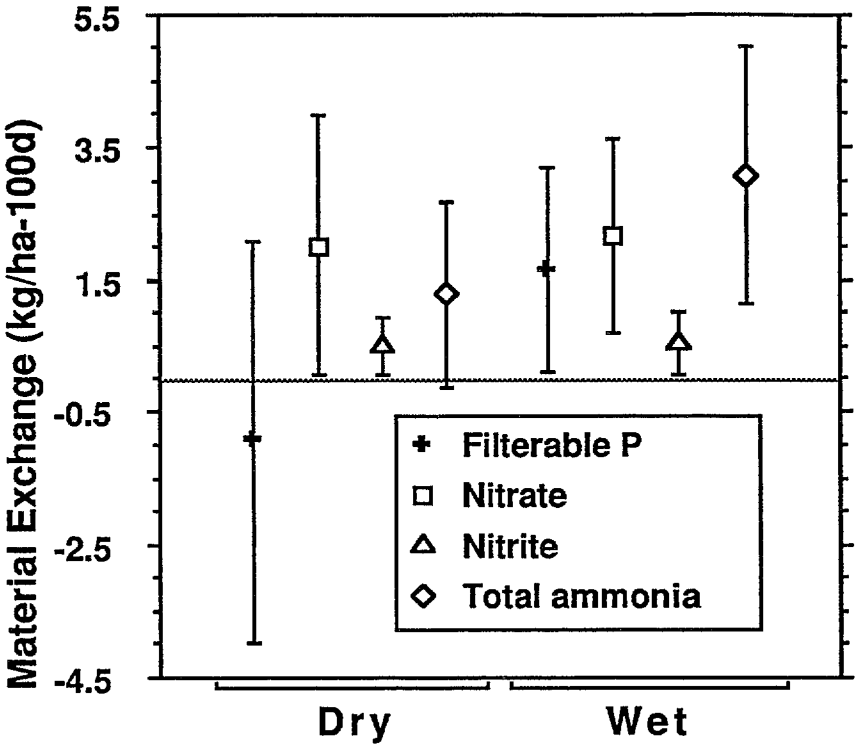
There were large seasonal differences for nutrient budgets. Production was significantly higher during the wet than dry season (Figure 10). The total quantity of feed added to ponds was not different between seasons. Therefore, the conversion of feed and protein to shrimp flesh was significantly more efficient during the wet season (Figure 10).

Phosphorus

Most phosphorus entered the pond with feed (54%), while water (44%) and fertilizer (2%) input accounted for the remainder (Figure 11). Shrimp accounted for less than 0.5% of total phosphorus input.

Table 3. Discharges of nitrogen and phosphorus from the Choluteca River and the shrimp farming industry (11,500 ha) in southern Honduras.

Variable	kg/year		
	Farms	River	Ratio (river/farm)
Total nitrogen	900,600	1,603,600	1.8
Total phosphorus	100,400	480,400	4.8



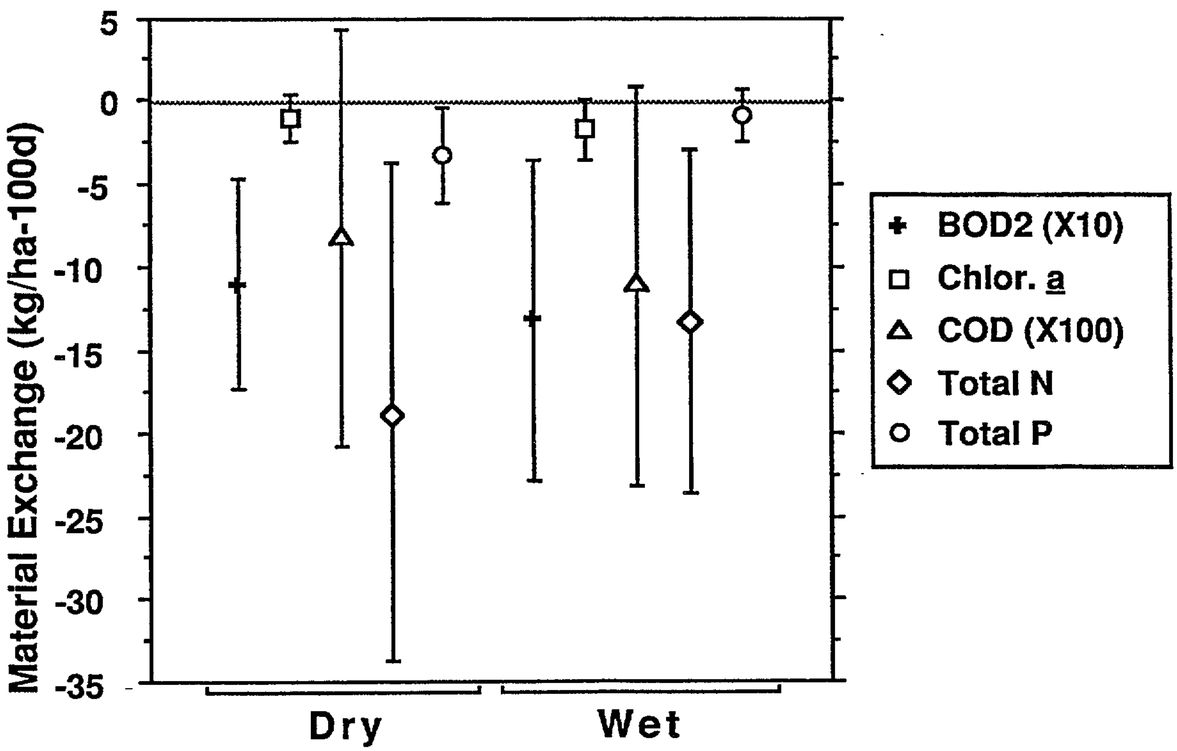


Figure 4. Mean net material exchange on all farms by dry or wet seasons. Negative values indicate a net discharge of material.

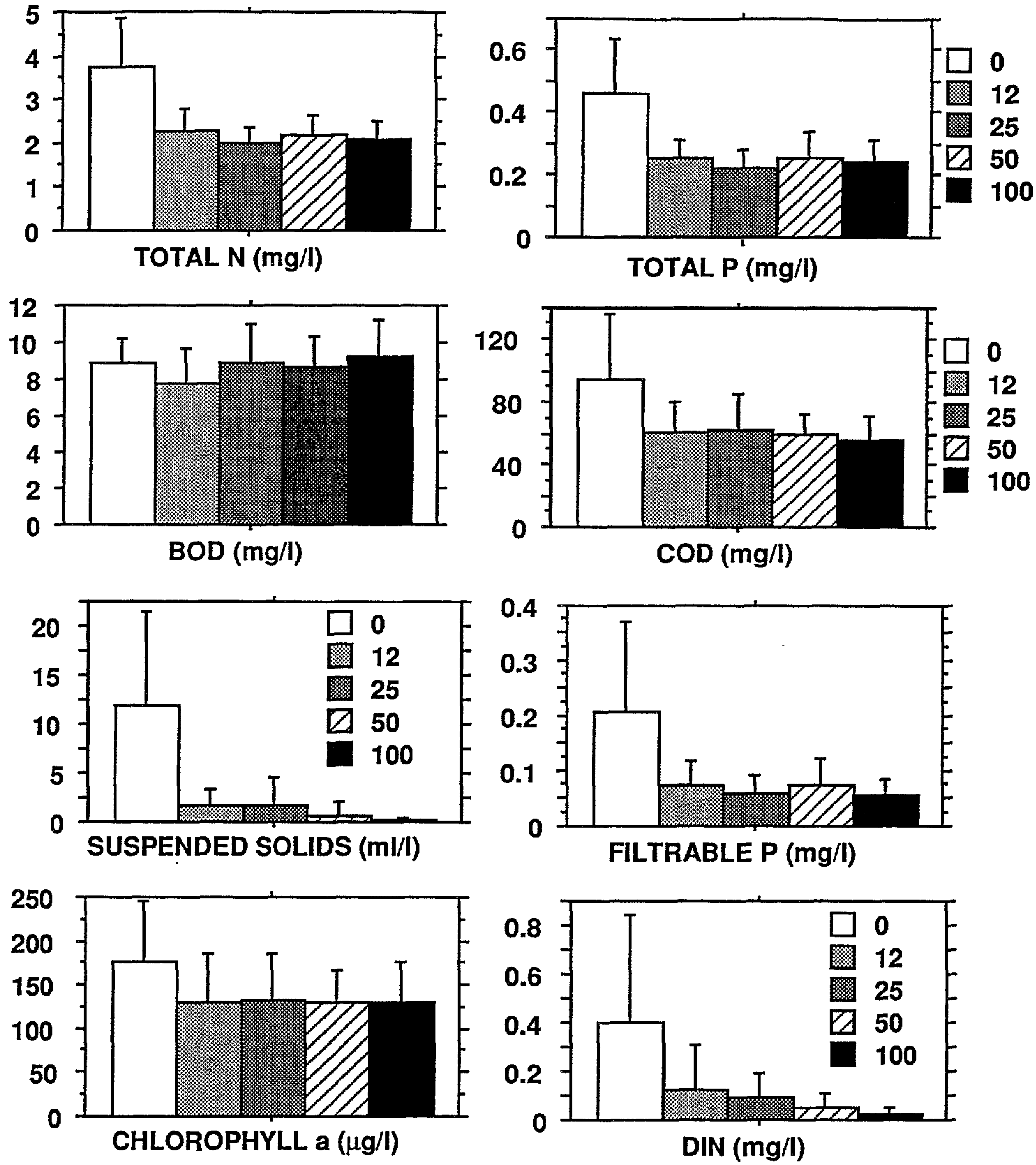


Figure 5. Mean effluent concentration of nutrients at 0, 12, 25, 50, or 100 % of pond volume during draining.

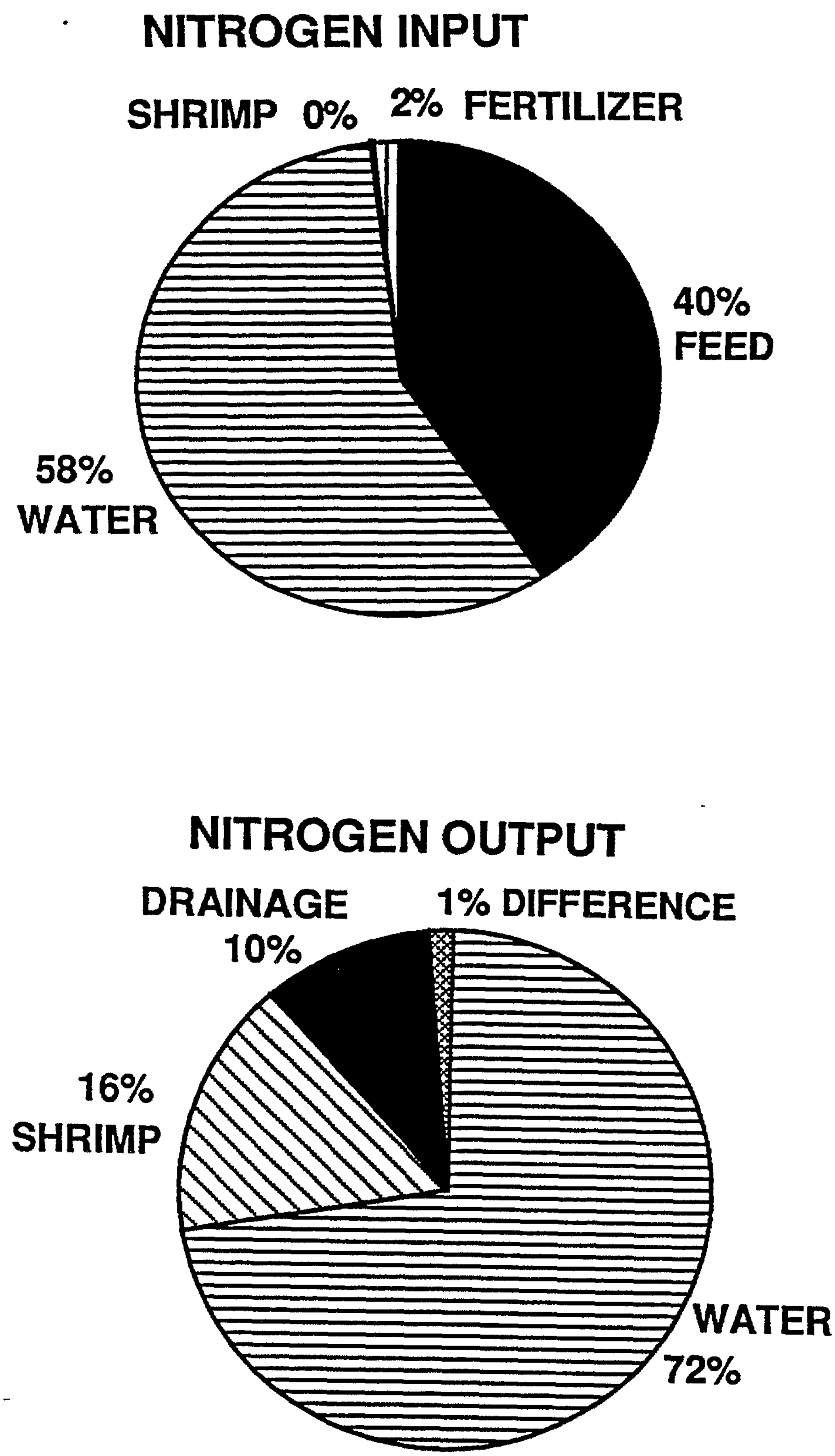


Figure 6. Mean nitrogen budget of shrimp ponds.

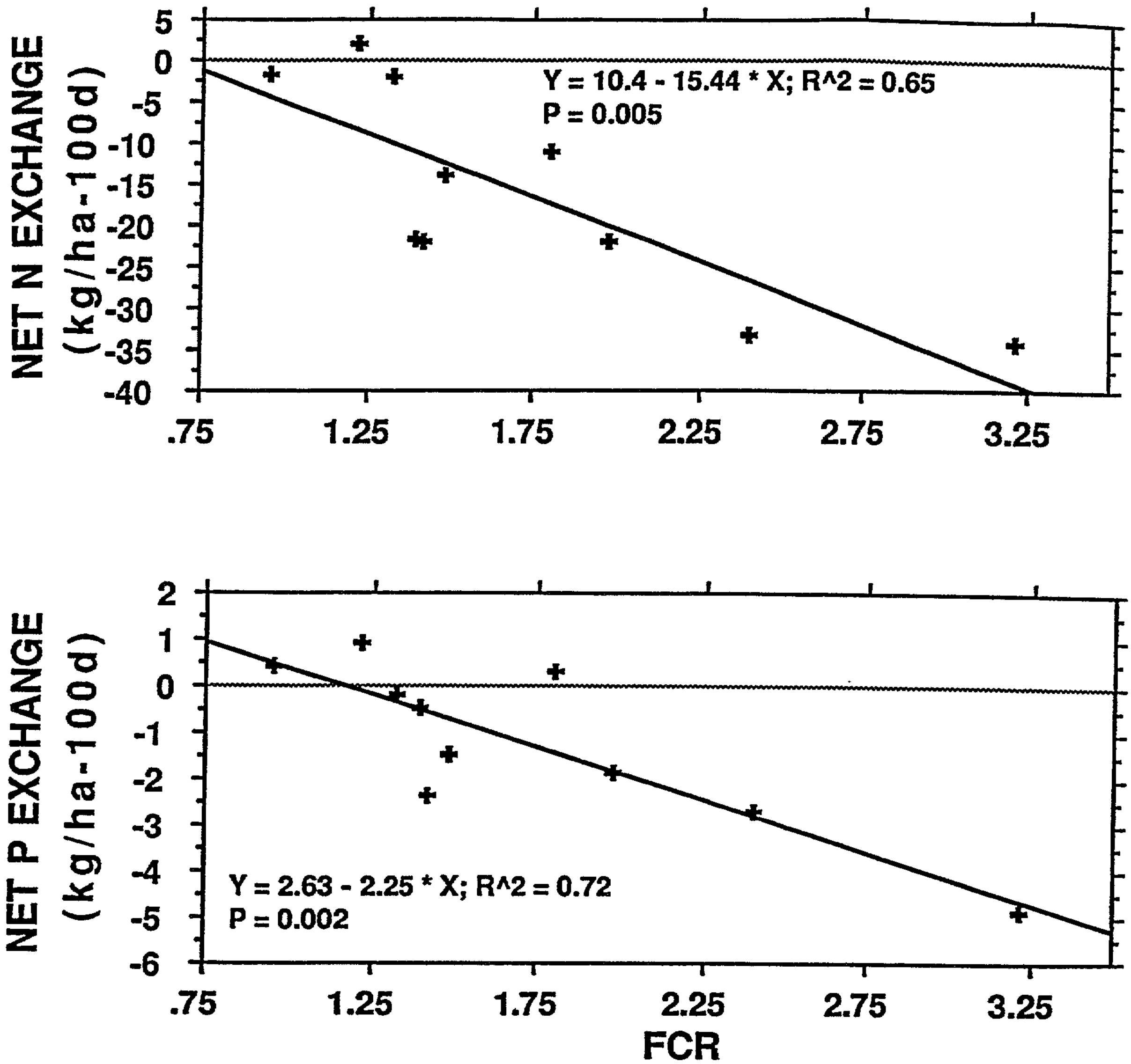


Figure 7. Net material exchange of nitrogen and phosphorus in relation to the feed conversion ratio. A negative exchange value indicates net discharge of material.

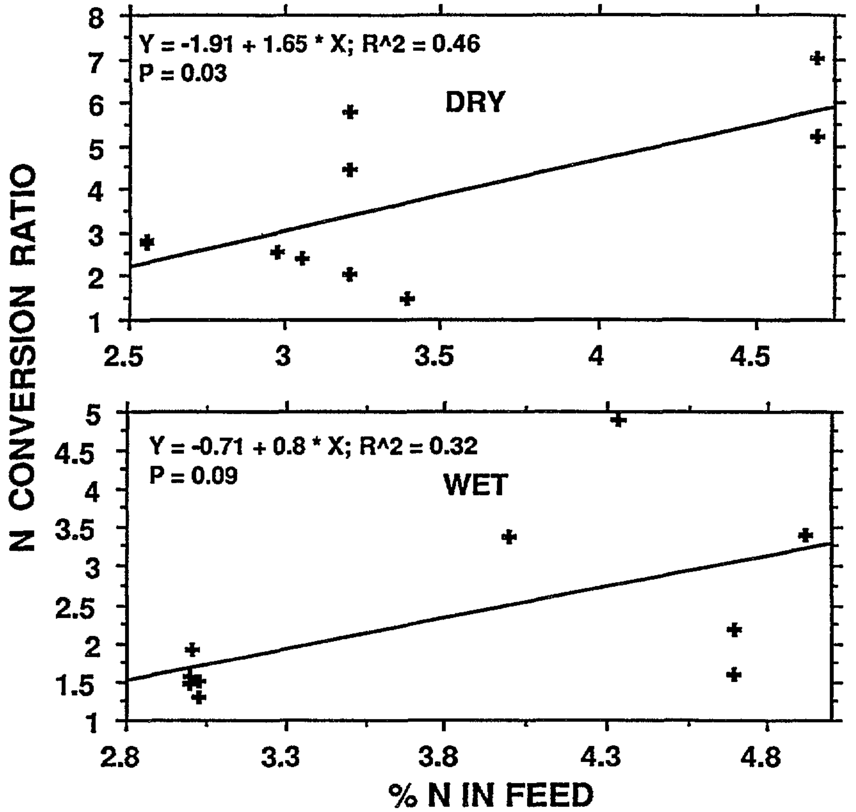


Figure 8. Conversion ratio of feed nitrogen to shrimp flesh in relation to percentage of nitrogen in the feed during dry and wet seasons.

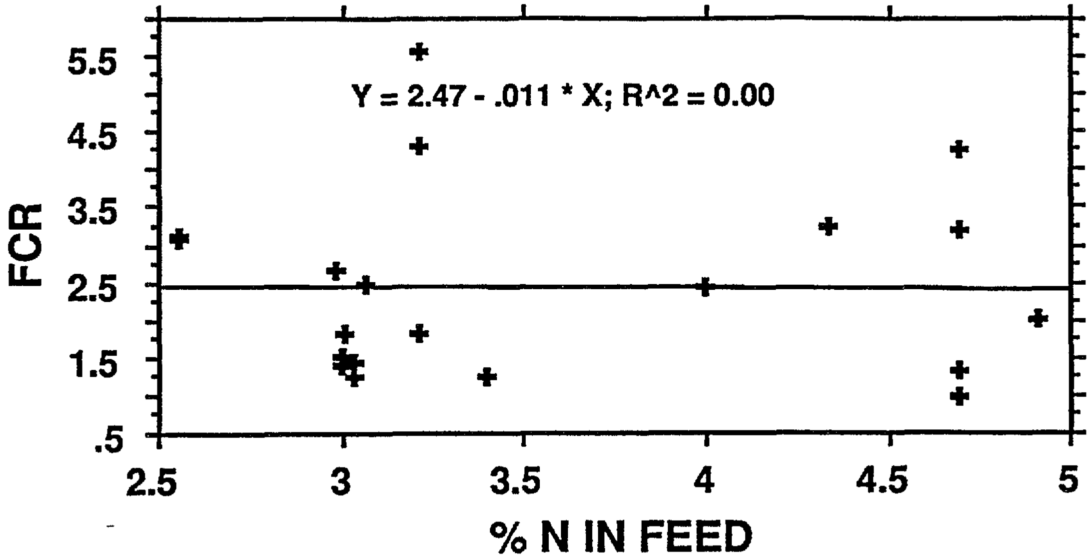


Figure 9. Feed conversion ratio in relation to the percentage of nitrogen in the feed.

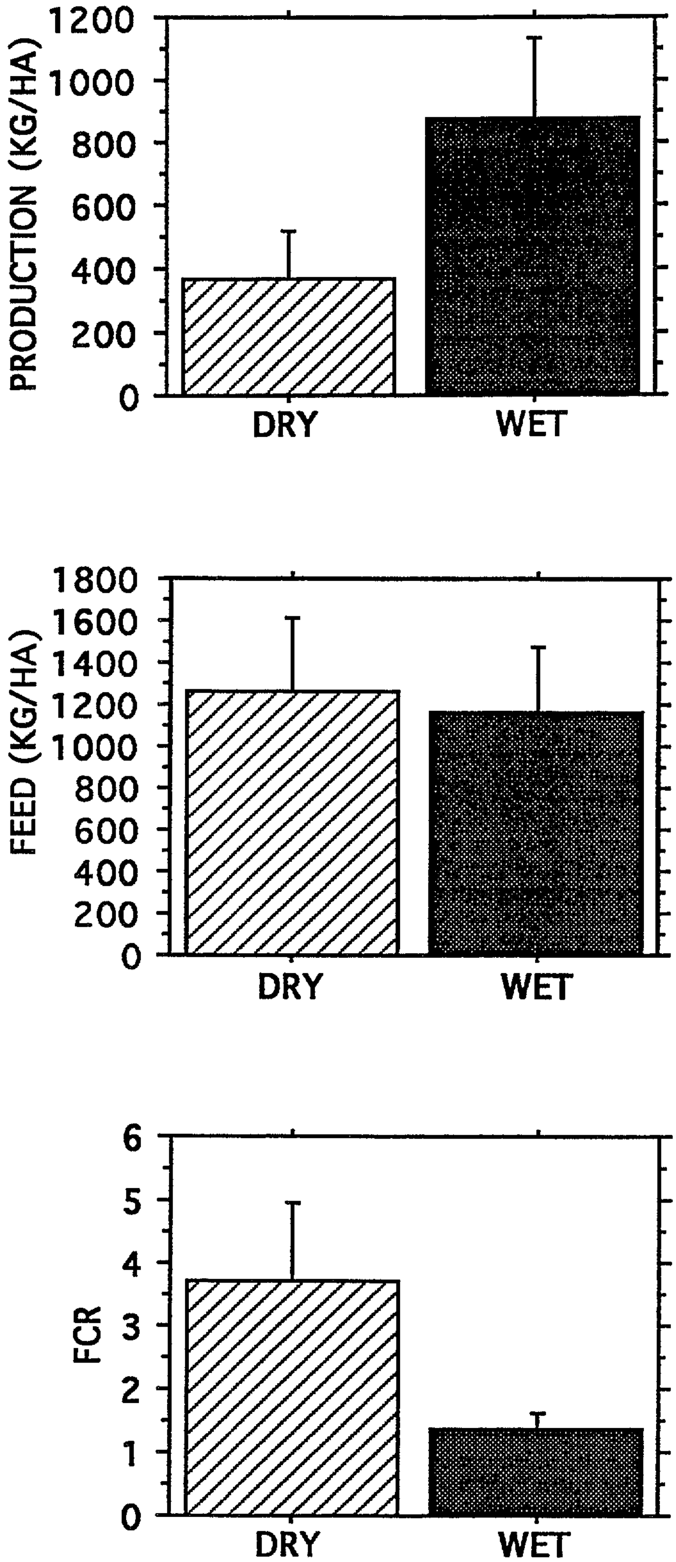
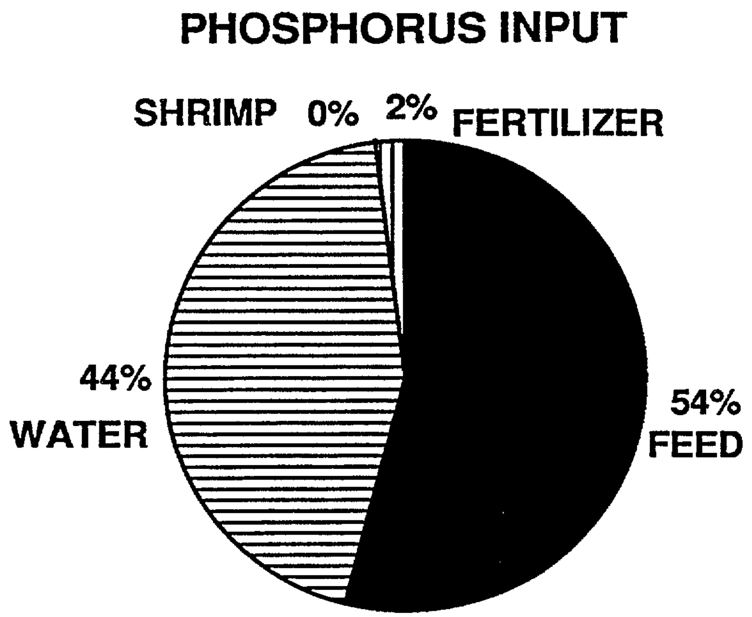


Figure 10. Mean shrimp production, total feed usage, and feed conversion ratios during dry and wet seasons. Bars indicate 95% C.I.



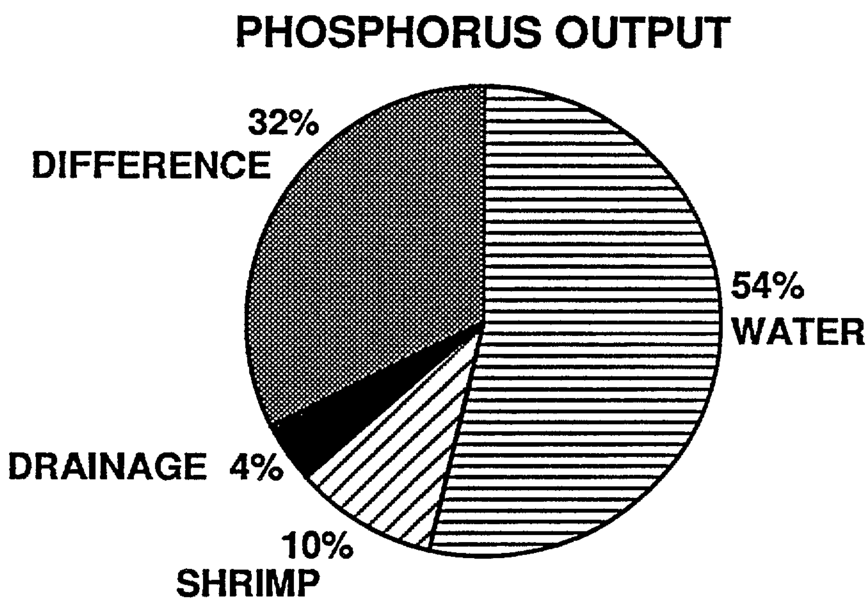


Figure 11. Mean phosphorus budget of shrimp ponds.

The majority of phosphorus was discharged from the ponds with daily water exchange (54%) and pond drainage (4%) Harvested shrimp accounted for 10% of phosphorus removal. Almost a third of input phosphorus was not observed in the sum of phosphorus discharged with water and harvested with shrimp, and was apparently fixed by the soils.

Mean conversion ratio of feed phosphorus to shrimp flesh was 6.3. Phosphorus conversion ratios were significantly greater (less efficient conversion) during the dry season (9.4) than during the wet season (3.7). Mean phosphorus conversion was not significantly different between type of estuary.

Recommendations

Much of the Honduran shrimp industry investment is from domestic sources, so a goal of the industry is to be sustainable for the next generation of Hondurans. The goal can be reached only if estuarine water quality is given prudent attention. Water quality depends on the assimilative capacities of estuaries and rate of nutrient discharge from farms. Farm nutrient discharges cannot exceed the assimilative capacities of the estuaries for sustainable production.

Estuarine assimilative capacities vary widely based on distance from the gulf, offshore currents, tidal fluctuation and estuarine hydrography (Ward and Montague, 1995). Hydrography of riverine estuaries in Central America is strongly influenced by seasonal rains, which cleanse estuaries during flooding. In Honduras, estuarine nutrient concentrations are significantly higher during the dry than wet season (Teichert-Coddington, 1995). Conditions become progressively worse with distance upstream from the gulf (Teichert-Coddington, 1995). The probability of low estuarine DO is higher during the dry season. Estuarine water quality is therefore more imminently critical, and of a lower assimilative capacity during the dry season.

Farm management practices can be modified to minimize nutrient discharge, particularly during the dry season when shrimp production is historically low (Teichert-Coddington et al., 1994). Inorganic fertilization can probably be eliminated in riverine estuaries, and reduced in embayment. Studies in Choluteca indicated that the use of inorganic fertilizers was of uncertain value during the wet season, and did not result in increased shrimp yield during the dry season (Green and Teichert-Coddington, 1990; Rodriguez and Teichert-Coddington, 1995). The current study demonstrated that inorganic nitrogen and phosphorus effluents were higher in fertilized than in unfertilized ponds. If fertilization increases nutrient discharges without increasing shrimp production, then estuaries will be needlessly enriched, and assimilative capacities will be more quickly reached at lower shrimp production levels. Dry season fertilization of ponds should therefore be stopped. A self-regulated moratorium on fertilizer use in riverine estuaries has, in fact, been employed by the Honduran National Association of Aquaculturists.

Feed accounted for 54% and 40% of nitrogen and phosphorus input, respectively, to ponds. Nitrogen appears to be a limiting factor to estuarine primary productivity, and should be controlled. This study indicated that nitrogen and phosphorus discharge increased with higher FCRs, and nitrogen discharge increased with higher feed protein levels. Yields of shrimp stocked at 5 to 10/m^2 were not different in Choluteca ($P > 0.05$) when using a 20% or 40% protein feed (Teichert-Coddington and Rodriguez, 1995). Low protein feeds should be used with semi-intensive shrimp culture under current feeding practices. Higher protein feeds have not demonstrated increased shrimp yields, but probably contribute to nitrogen discharge.

The current study indicated that mean dry season production was significantly lower than mean wet season production, yet similar quantities of feed were used during both seasons. Feed conversion ratios and wastes were consequently higher during the dry season. These data indicate that mean feeding rates had not been adjusted during the dry season to reflect lower dry season shrimp growth. Rodriguez and Teichert-Coddington (1995) decreased feeding rates by half during a dry season trial without any impact on shrimp production. Current studies are focusing on the combination of higher feed protein and lower feeding rates to reduce total nutrient effluents.

Other management practices to reduce nutrient wastes in semi-intensive culture should be tested. Feeds with longer water stability are being employed sporadically, but controlled studies on the benefits of these feeds are unknown. Pond designs may have to be altered to gain further reductions in nutrient discharge. Mean area of the ponds in this study was 21 ha. It is hard to sample shrimp populations and distribute feeds efficiently in ponds of this large size.

Smaller ponds would be more expensive to build, but the savings in feeds, and reduction of nutrient discharge might make smaller pond designs more sustainable.

Acknowledgments

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Relationships among Stocking Density, Survival and Yield in Ponds Affected by the Taura Syndrome during Wet and Dry Seasons in Honduras

Work Plan 7, Honduras Study 3C

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Introduction

Relationships among density, mean size, survival and yields of semi-intensively cultured shrimp are not well understood. Shrimp size at harvest may not be proportional to density, and survival of stocked shrimp usually decreases with increasing density. Yields are usually linearly related to survival, but survival is unrelated to treatment. Results of management strategies, especially those related to feeding and growth, are not highly predictable. A better understanding is needed to increase production efficiencies and lower nutrient effluents.

The original objective of this study was to evaluate the relationships among stocking density, mean shrimp size, survival and yield. However, the Taura Syndrome (TS) struck the area after stock-out of the wet season study, severely lowering survivals. Producers naturally became interested in the effect of stocking rate and season on survival under TS conditions. Our study results were therefore appropriated to that end.

Materials and Methods

A completely randomized design was used to test 4 stocking densities of *Penaeus vannamei* in earthen ponds during wet and dry seasons of the year. Juvenile shrimp were stocked at 6, 8, 10, or 12/m^2. The design was tested on two farms during the wet season and on three farms during the dry season. Each treatment was replicated 4 times during the wet season, and 3 times during the dry season on Farm A. On Farm B, each treatment was replicated 2 times during the wet season and only once during the dry season. Farm C stocked one pond at each stocking density.

Shrimp stocked in ponds of all farms had been raised in nurseries from the same source of hatchery-spawned postlarvae. An extra 15% of stocking rate were added to ponds to account for stocking mortality.

During the wet season, commercial grow-out ponds on both farms were used. These ranged from 17 to 36 ha in area. During the dry season, nursery ponds ranging in size from 0.7 to 1.4 ha were stocked. Because of their large size, all wet season ponds could not be stocked simultaneously. Rather, one replicate pond of each treatment was stocked simultaneously in blocks over a period of several weeks. During the dry season, ponds on each farm were stocked simultaneously. Wet season ponds were stocked during 13 June to 20 July 1994 on Farm A, and during 19 July to 9 August 1994 on Farm B. Farm A ponds were harvested after 92 to 107 d, and Farm B ponds were harvested after 95 to 101 d. Dry season ponds were stocked between 27 December 1994 and 12 January 1995 and harvested after 95 to 99 d.

Shrimp were fed an ALCON 20% protein diet based on 75% of feeding tables that decreased the feeding rate as mean shrimp weight increased. The rate adjustment was generally described by the following equation: $Y = 11.74 - 6.79 \text{ Log}_{10}X$, where Y = % of shrimp biomass, X = mean shrimp weight. The feeding tables assumed a weekly mortality of 1.2% during the wet season and 4.2% during the dry season. Feeding rates were calculated for each pond separately during the wet season, and equally for all ponds of a given treatment during the dry season.

Table 1. Production of *Penaeus vannamei* stocked at 4 rates on each of two farms during the wet, and three farms during the dry season of the year in southern Honduras.

Farm	Number of ponds	Stocking density (No./m^2)	Total production (kg/ha)	Mean weight (g)	Survival (%)	Feed conversion (Feed/yield)	Net income ($/ha)
				Wet season			
A	4	6	502	16.7	50.5	2.13	3603
	4	8	616	13.7	57	2.38	3795
	4	10	658	13.9	49.5	2.69	3879
	4	12	757	11.9	52.5	2.55	3986
		Mean	633	14.1	52.4	2.43	3816
B	2	6	488	17	45.8	2.28	3404
	2	8	595	17.4	42	2.65	4408
	2	10	699	16.6	42.3	2.51	4664
	2	12	855	15.7	44.5	2.62	5522
		Mean	659	16.7	43.6	2.51	4500
				Dry season			
A	3	6	290	8.8	46	2.67	1169
	3	8	270	8.6	40	3.61	801
	3	10	334	7.6	43	3.8	53
	3	12	315	9	29	4.76	598
		Mean	302	8.5	39.5	3.71	655
B	1	6	303	7.3	69	2.29	611
	1	8	335	6.8	62	2.8	339
	1	10	427	6.7	64	2.75	339
	1	12	523	5.4	81	2.51	321
		Mean	397	6.6	69	2.59	402
C	1	6	253	7.4	57	1.34	340
	1	8	202	6.5	39	2.09	-61
	1	10	303	6.2	49	2.29	-189
	1	12	238	4.9	41	2.01	-514
		Mean	249	6.3	46.5	1.93	-106

Similar feeding rates were used on the different farms, but actual amounts fed on each farm were calculated separately. Shrimp were sampled by cast net each week from a pre-determined sampling grid for each pond. Feeding rates were adjusted based on weekly shrimp samples. Daily feed was equally divided into morning and afternoon rations (morning and afternoon).

Water was generally exchanged according to the following schedule: the first 2 to 4 weeks no exchange and thereafter, daily exchange at 5% of pond volume. During the dry season, Farm A exchanged water at 5% of pond volume once a week, instead of daily. Water was exchanged by first draining, and then by refilling. Record was kept of exchange frequency and volume.

A limited economic analysis was performed on the data. Variable costs were the sum of seed and feed costs. Total revenue was calculated by pond from the total harvested weight and the price received at the packing plant. Prices varied greatly by shrimp size, so calculations employed a price based on the mean harvested shrimp weight per pond. Juvenile seed and feed cost $9.23/1000 and $0.24/kg, respectively. Shrimp prices per kg of tails ranged from $9.25 to $7.14, decreasing as the mean size decreased.

Water quality variables were monitored to form nutrient budgets for ponds with different stocking rates. Water was analyzed for total settleable solids (American Public Health Association (APHA) et al., 1992), nitrate nitrogen by cadmium column reduction to nitrite (Parsons et al., 1992), total ammonia nitrogen (Parsons et al., 1992), filterable reactive phosphate (Grasshoff et al., 1983), chlorophyll *a* (Parsons et al., 1992), total alkalinity by titration to 4.5 pH endpoint, salinity, and 2-d and 7-d BOD at ambient temperature. Total nitrogen and total phosphorus were determined by nitrate and phosphate analysis, respectively, after simultaneous persulfate oxidation (Grasshoff et al., 1983). Organic phosphorus was calculated from the difference of total phosphorus and filterable reactive phosphate. Dissolved inorganic nitrogen (DIN) was the sum of nitrate, nitrite and total ammonia nitrogen (TAN), and organic nitrogen was the difference of total nitrogen and DIN.

Table 2. Mean production of *Penaeus vannamei* during wet and dry seasons of 1992 to 1994 . Results are means of 3 to 4 replications. Data were collected from the same farm, using 100% hatchery reared animals.

Year	Stocking density (No./m^2)	Production (kg/ha)	Mean weight (g)	Survival (%)	Harvest density (No./m^2)
		Wet Season			
1992	5	1430	24.8	98	4.9
1992	10	2100	21.5	87	8.7
1993	7.5	1258	18.1	96	7.2
1994	6	488	17.0	46	2.8
1994	8	595	17.4	42	3.4
1994	10	698	16.6	42	4.2
1994	12	855	15.7	45	5.4
		Dry Season			
1992	5	419	9.6	89	4.5
1992	10	597	9.7	62	6.2
1993	7.5	360	6.7	74	5.6
1994-5	6	303	7.3	69	4.1
1994-5	8	335	6.8	62	5.0
1994-5	10	427	6.7	64	6.4
1994-5	12	523	5.4	81	9.7

Data were analyzed by ANOVA and linear regression (Haycock et al., 1992). Except where indicated, data from both farms were pooled. Analyses were separate by season. Differences were declared significant at alpha = 0.05.

Results

The Taura Syndrome did not intensify its effects on shrimp during the dry, cool season, as anticipated. Mean wet and dry season survival for pooled data on Farms A and B was 44% and 69%, respectively (Table 1). Survivals during wet and dry seasons would normally have been about 94% and 75%, respectively (Table 2).

Wet season

There was no significant correlation between shrimp stocking density and survival when farm data were pooled (Figure 1). Survival for both farms fell within the same range. Production increased linearly with an increase in stocking density (Figure 2).

Mean shrimp size decreased significantly, with an increase in stocking density on Farm A, but not on Farm B (Figure 3). Net income significantly increased with stocking density on Farm B, but not on Farm A (Figure 4).

Dry season

There was no significant correlation between stocking density and survival (Figure 1). Mean survival differences among farms were greatest at the highest density (Figure 1), where mean survivals at Farms A (39.5%) and C (46.5%) were notably lower than mean survival at Farm B (69%).

Shrimp production increased linearly with an increase in stocking density (Figure 2) when farm data were pooled. However, analysis by farm indicated that the correlation was significant only for Farm B. Production at Farms A and C indicated insignificant change with increasing density.

Mean shrimp size significantly decreased with an increase in stocking density on Farms B and C, but not on Farm A (Figure 3). Net income was not significantly correlated with stocking density at Farms A and B, but significantly decreased with density on Farm C (Figure 4).

Water quality

Analyses of water quality and nutrient budgets are not complete. However, preliminary data indicate that, with minor exceptions, increasing the stocking density from 6 to 12/m^2 had insignificant effect on the export of nutrients from ponds.

Discussion

The Taura Syndrome reduced shrimp survival below normal at all farms during both growing seasons. Mean survivals during the past two years of work in Honduras (Teichert-Coddington and Rodriguez, 1994; Teichert-Coddington and Rodriguez, 1995) ranged from 87 to 98% during the wet seasons, and from 62 to 89% during the dry seasons (Table 2). Mean survivals during the current experiment (Table 1) were almost 50% and 30% lower during the wet and dry seasons, respectively. The incidence of Taura was expected to increase with stocking density, because of the increased frequency of contact among animals. Survival normally decreases at higher densities, even without TS (Teichert-Coddington and Rodriguez, 1995). Results of these experiments indicated no abnormal survival because of TS during either season on any farm. It is possible that stocking rates exceeding 12/m^2 could result in higher mortalities, but such densities are not used in semi-intensive systems characteristic of Honduras.

Seasonal production differences were large as always (Teichert-Coddington et al., 1994). Mean dry season yield for Farms A and B was 349 kg/ha, about half of wet season yield (646 kg/ha). Dry season yield was about normal, but wet season yield was about half of normal. There are concerns that TS affects growth of survivors, in addition to inducing mortality of juveniles. Lesions can be found on adult shrimp, indicating that the disease is present, but subacute. However, the low yields in the current experiment were more likely related to the abnormally low survivals from the Taura Syndrome. Yield reductions in past experiments have been correlated with low survival, probably because growth of surviving animals didn't increase enough to compensate for loss in numbers.

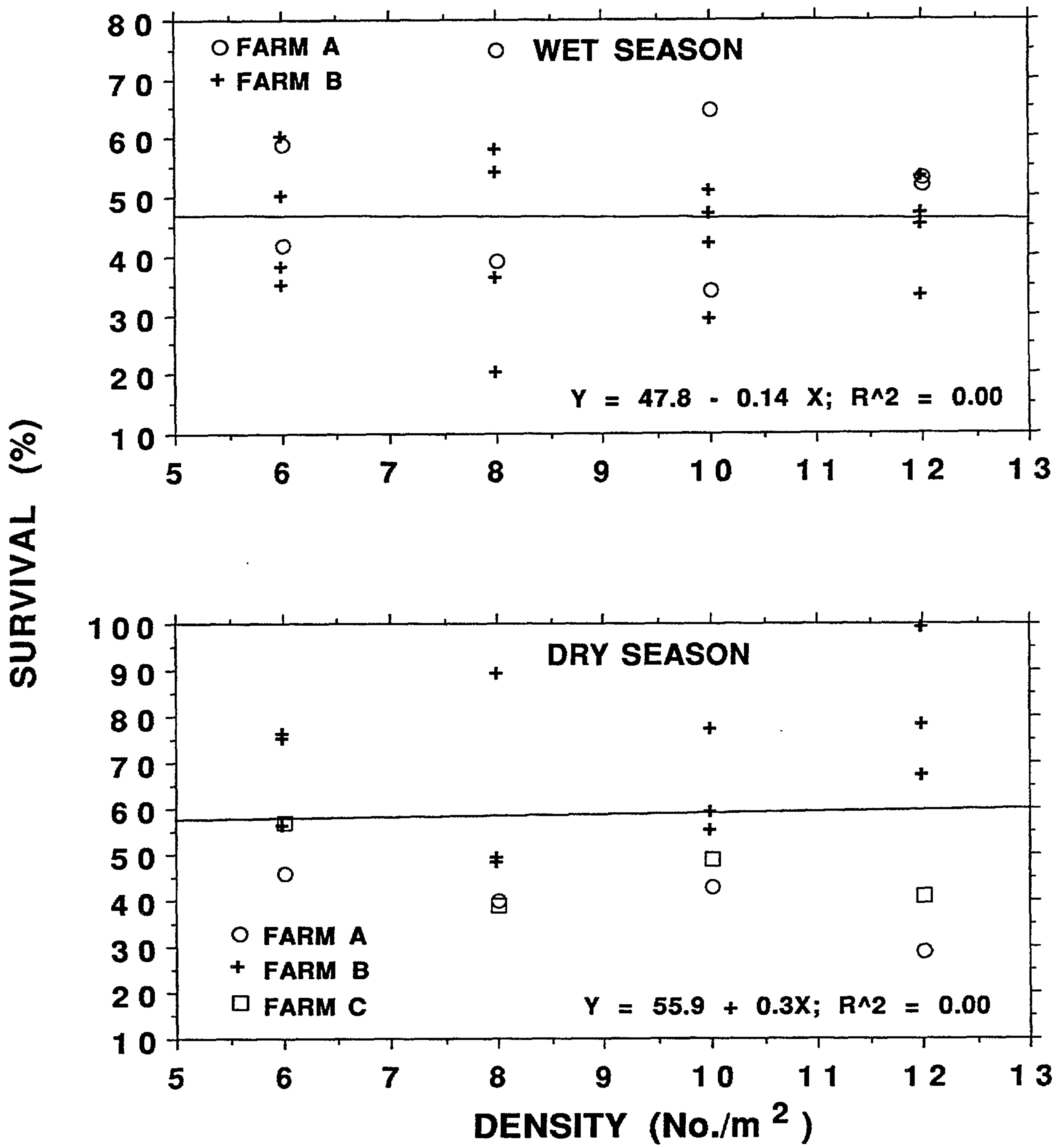

Figure 1. Relationship between shrimp survival and stocking density on different farms and during wet and dry seasons in southern Honduras.

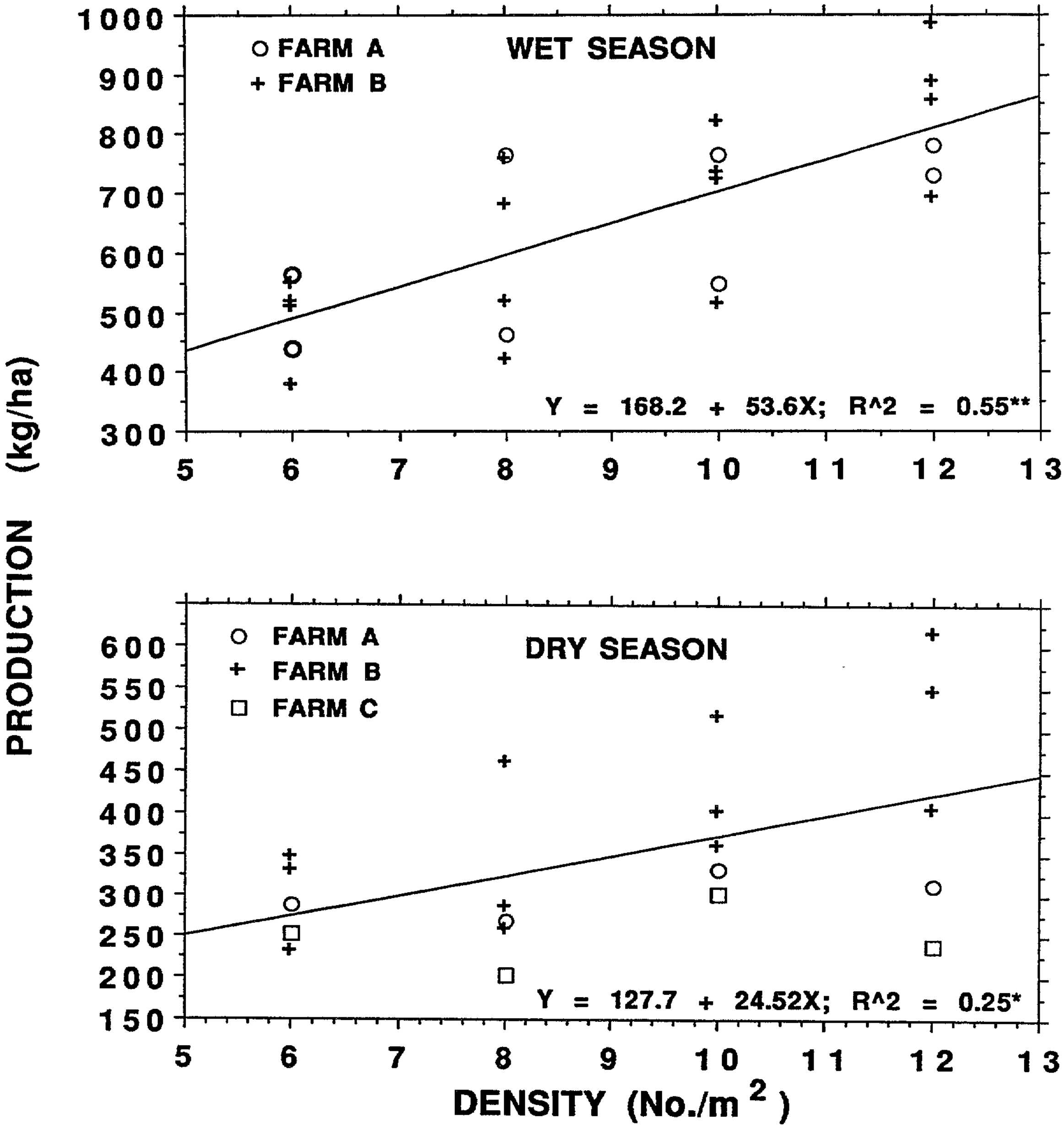

Figure 2. Relationship between shrimp production and stocking density on different farms and during wet and dry seasons in southern Honduras [* significant ($P < 0.05$); ** very significant ($P < 0.01$)].

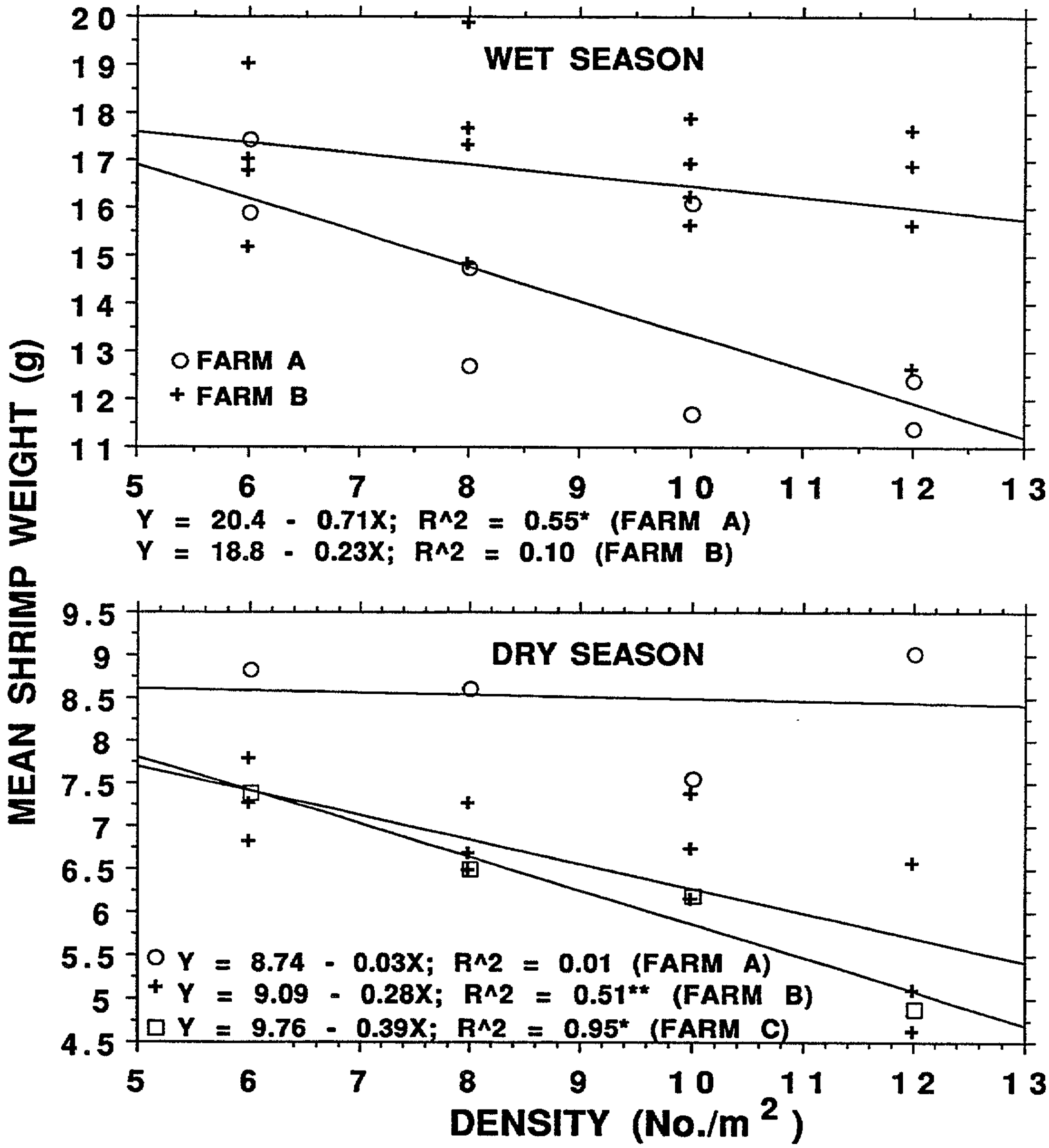

Figure 3. Relationship between mean shrimp weight and stocking density on different farms and during wet and dry seasons in southern Honduras.

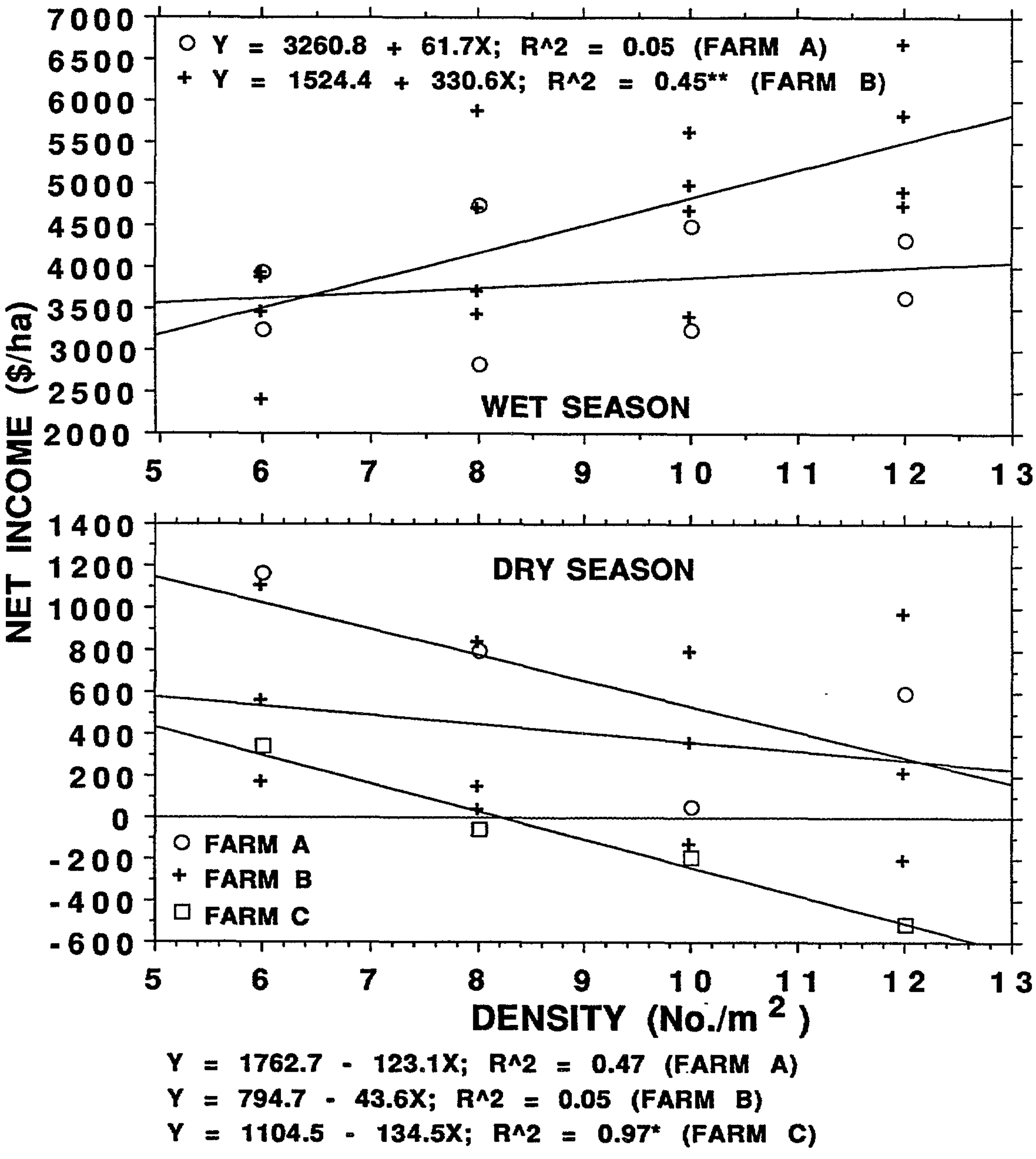

Figure 4. Relationship between net income and stocking density on different farms and during wet and dry seasons in southern Honduras [* significant ($P < 0.05$); ** very significant ($P < 0.01$)].

An objective of this experiment was to evaluate the effect of stocking rate on mean harvest size of shrimp, because growth reduction commonly occurs in cultured animals when stocking rates are increased. Past experiments (Teichert-Coddington and Rodriguez, 1995) indicated that stocking rate had only slight impact on wet season shrimp size and no impact on dry season shrimp size. In the current experiment, stocking rate was only sometimes related to mean harvest size. The results were likely affected by TS, but survivals, albeit abnormally low, were similar across all stocking densities. During the wet season, mean weight of shrimp at Farm A, but not at Farm B, decreased about 0.7 g per animal stocked above $6/m^2$. A dry season reduction of 0.3 to 0.4 g per stocked animal occurred at Farms B and C, but not at Farm A. These data indicate that predictions of harvest size based on stocking rate can not be confidently made. Results are changeable by season and farm. Much more research needs to be done to make management of stocking rates for a particular harvest size more predictable.

Income is related to both biomass and shrimp size, because the price per unit weight of shrimp increases with size. The relationships among stocking rate, yields and harvested mean shrimp size is economically important. On Farm B where most replication of treatments occurred, net income increased with density during the wet season because production increased without a decrease in harvest size. However, dry season net income remained unchanged with increased density despite an increase in production, because mean shrimp size decreased. The loss in size economically compensated the increase in production. Farm A net income showed less correlation to density increase during the wet season, despite increasing production, because mean shrimp size decreased with density. Farm C net income decreased with increased density during the dry season, because mean size decreased while production remained unchanged.

There are strong seasonal effects on both production and net income. The seasonal effects on production are compounded by estuarine eutrophication during the dry season because of reduced estuarine exchange with the Gulf of Fonseca (Teichert-Coddington, 1995). The only way to combat the eutrophication is to reduce farm nutrient effluents to the estuaries. The following processes have been demonstrated during the dry season: shrimp yields decrease drastically; net income decreases with increased stocking density; estuarine water quality significantly degrades. It follows that dry season management should be different from wet season management. Dry season stocking rates might better be minimized to reduce nutrient inputs and outputs, variable costs and economic risk.

The results from this study suggest some management guidelines for ponds affected with TS. Stocking rates can be safely increased to compensate for poor survival, although the cost of additional seed will obviously reduce profits. During the wet season, each farm should determine the number of shrimp that need to be harvested per unit area to be profitable, and over-stock with sufficient animals to account for previously estimated survivals. Mean harvest weight can be manipulated by duration of production cycle. During the dry season, ponds probably should be managed with minimal inputs. Stocking rates should be closely analyzed, because of reasons given above, and because increased seed costs will signify a greater proportion of total variable costs.

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East Africa

The continuing unrest in Rwanda has forced the CRSP to close its research site there. The losses, in terms of the deaths, disrupted lives, and lost expertise of both the professional staff and the area farmers, are immeasurable. In an effort to minimize the overall loss to the region and to the aquaculture community, the CRSP has been actively engaged in selecting a new site from which to build regional capacity in aquaculture research. The selection process has entailed much research, several site visits, extensive correspondence, laboratory analyses of soils and water samples, and other exploratory efforts, and the process is still underway. Site selection criteria have been developed, data have been collected from several sites, promising sites have been evaluated, and work has begun on a characterization of African soils. A final determination will be made by the time the Continuation Plan commences.

In addition to soil studies, members of the Africa team at Auburn are investigating the effect of temperature on appetite and growth response of tilapia fry. The results of the study will enable researchers to investigate the effects of growth rate on the timing of gonadal differentiation and the efficacy of sex reversal, leading to more efficient use of hormone-treated feed. Preliminary studies have not yielded sufficient data, and further trials are planned to obtain the needed data points.

It is an indicator of the resiliency of the collaborative research process that research experiments are portable among sites. As an example, researchers noted that worldwide, red tilapia have generally been perceived by producers as having greater consumer acceptance, although existing research indicates that the growth rate of Nile tilapia is superior. In trials currently underway, Auburn researchers working at the El Carao Fish Culture Station in Comayagua, Honduras, are investigating the reproductive efficiency of Nile tilapia and red tilapia, and their comparative growth and efficacy of sex reversal. The work is being carried out at the El Carao Station, but the impact of the results will be important for tilapia farmers throughout the world. Researchers at El Carao are also investigating the growth and efficiency of sex reversal of Nile tilapia that are fed hormone-treated feed stored under different storage regimes, another study that was originally programmed for the Rwanda site.

Work at OSU continues the biotechnological research focus of the Egypt project by examining the efficacy of a short-term immersion procedure for masculinizing tilapia. Two synthetic androgens, 17α-methyldihydrotestosterone (mestanolone) and 17α-methyltestosterone, were evaluated at two concentrations, using 3-hour exposures at 10 and 13 days after fertilization. Results indicate that short-term immersion in 17α-methyldihydrotestosterone at a concentration of 500µg/l shortens the treatment period, thereby reducing possible worker exposure to anabolic steroids.

Masculinization of Nile Tilapia (*Oreochromis niloticus*) through Immersion in 17α-Methyltestosterone or 17α-Methyldihydrotestosterone

Interim Work Plan, Africa Study 2

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Introduction

All-male populations are used in tilapia aquaculture because mixed sex populations often undergo precocious maturation, which shunts energy to gonadal rather than somatic growth, and early reproduction, which leads to the harvest of many unmarketable fry. Furthermore, all-male populations are desirable, because males grow larger than females.

One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion. This involves the administration of synthetic androgens (to produce male populations) or estrogens (to produce female populations). Several methods of steroid administration are possible, including injection, microencapsulation, feeding of steroid, and immersion of fry in steroid solutions. The latter two are non-invasive and therefore, the most practical for application to aquaculture.

The use of steroid-treated feeds for the production of all-male populations is widespread in tilapia aquaculture. Conversely, the use of immersion techniques is not fully developed for practical use. Torrans et al., (1988) successfully masculinized blue tilapia (*O. aureus*) by immersion in the synthetic androgen mibolerone (MB; 17-hydroxy-7,17-dimethylestr-4-en-3-one). The optimum conditions for treatment were a five-week immersion period at 600 µg /l, with steroid replacement weekly. Pandian and Varadaraj (1987) masculinized Mozambique tilapia (*O. mossambicus*) by immersion in 17α-methyl-5-androsten-3ß-17β-diol (5 or 10 µg/l). The immersion period lasted 10 days, beginning at 10 days post fertilization. Although the authors reported 100% masculinization, detailed information regarding temperature, type of culture system used, fish density, and frequency of water exchange during the immersion period was not included. These omissions make the replication and future application of this research difficult.

In salmonid aquaculture, a short term immersion of fry in a solution of 400 µg 17α-methyltestosterone (MT; 17-hydroxy-17-methylandrost-4-en-3-one)/l for a period of 2 hrs successfully produces all-male populations (Piferrer and Donaldson, 1989; Feist et al., 1995). Methyltestosterone is one of the most commonly used sex inverting agents, but is susceptible to aromatization and has been associated with paradoxical feminization in coho salmon (*Oncorhynchus kisutch*; Piferrer and Donaldson, 1991). Paradoxical feminization can be avoided by use of a nonaromatizable androgen such as 17α-methyldihydrotestosterone (Piferrer et al., 1993).

The objective of this research was to develop a short term immersion procedure for the masculinization of Nile tilapia (*O. niloticus*). Compared to feeding methods, immersion reduces human handling of steroid and provides more uniform exposure of fish to steroid. Two synthetic androgens were used, MT and 17α-methyldihydrotestosterone (MDHT; 17ß-hydroxy-17-methyl-5α-andro-stan-3-one). Methyldihydrotestosterone is also known by the trade name mestanolone.

Materials and Methods

Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of HPLC grade methanol (10 mg/ml). Breeding families (one male to three females) were placed in 208-L aquaria. The temperature was maintained at 28-30°C. Breeding activity was

Table 1. Sex distribution, mortality data, and sample size (n) from experiment one (EX I) and two (EX II). Group abbreviations are as given in Fig. 1.

Group	EXI				EXII			
	males	females	Mortality (%)	n	males	females	Mortality (%)	n
MT-100	30	11	58	41	12	17	26	29
MT-500	16	15	46	31	16	14	64	30
MDHT-100	33	13	53	46	15	14	33	29
MDHT-500	36	0	63	36	31	2	33	33
ETH	18	23	59	41	14	18	22	32
CTL	7	12	81	19	17	34	35	51
FED	23	2	62	25	---	---	---	---

monitored daily. Once breeding occurred, the other fish were removed and the brooding female left to incubate the progeny. At 10 days post fertilization (DPF), fry were removed from the female and assigned to experimental groups (n=100/group). Each group was housed in a separate 3.8-L glass jars with 3 L of fresh water. The water was maintained at 28 ± 2°C under constant aeration. Treatment consisted of a 3 hr immersion on 10 and 13 DPF. Steroid was evaporated under N_2 (g) and delivered in 0.5 ml of ethanol. Steroid was allowed to mix by aeration for 30 min before addition of fry. Fry were immersed in MT or MDHT at 100 or 500 µg/l (MT-100, MT-500, MDHT-100, MDHT-500). Control groups included the following: immersion in water and ethanol vehicle (ETH), an immersion in water alone (CTL), and water immersion followed by feeding of MT-treated diet (FED; 60 mg/kg) from 10 to 30 DPF. After each immersion, the fry were collected and placed in new jars that contained fresh water. The first experiment (EX I) was replicated (EX II) with omission of the FED group. In EX I, the groups were held in the jars (3.8 L) until the end of the feeding treatment period (30 DPF). In EX II the groups were held in the 3.8-L jars only until after the 13 DPF immersion. The groups in both experiments were transferred into separate 20-L chambers for grow-out in a recirculating system. Water temperature in the grow-out system was maintained at 28 ± 2°C. At 100 DPF, sex ratios were determined by examination of *in situ* (40X) and squash (100X) preparations after aceto-iron hematoxylin (Wittman, 1962) staining. The weights and total lengths of sampled fish were recorded (EX II) at this time.

Sex ratio data were analyzed using the chi-square test (a<0.05; Zar, 1984). The CTL and ETH groups were not significantly different, and were pooled for comparison to other groups. The mean final weights of sampled fish from EX II were analyzed for differences between groups using one-way ANOVA (a<0.05). Mortality data were analyzed using the chi-square test (a<0.05; Zar, 1984).

Results

Immersion in MDHT at 500 µg/l resulted in 100 (EX I) and 94 (EX II) percent male populations (Fig. 1). In EX 1, MT and MDHT treatments at 100 µg/l resulted in significant skewing of the sex distribution toward males (73 and 72 percent male, respectively); however in EX II, the proportion of males were not significantly different from controls (Table 1). Methyltestosterone at 500 µg/l had no masculinizing effect in either experiment. The MT feeding treatment resulted in 92 percent males (EX I).

Mortality was not significantly different between treatment groups in EX I (excluding CTL). The higher mortality observed in the CTL group in EX I was likely due to a clogged inlet that restricted water flow for about 24 hr. The ethanol treated and control groups in EX II, had significantly different mortality; however, the remaining groups were not different from either ETH or CTL. The MT-500 group suffered higher mortality due to cannibalization by an adult fish that jumped from a neighboring tank. No significant differences between treatments in weights or lengths were detected in EX II.

Discussion

Immersion treatment on 10 and 13 DPF with MDHT at a concentration of 500 µg/l successfully masculinized Nile tilapia. Conversely, MT at similar levels did not significantly alter the sex ratio. Lack of an effect in the MT treatment (500 µg/l) may be due to conversion of MT to a less active form, or simply to a higher rate of clearance from the body than MDHT. Another possible explanation for the differing effects of the two steroids is that MDHT is a more potent masculinizing agent than MT. Piferrer et al., (1993) found that MDHT was twice as potent as MT in masculinizing female chinook salmon (*Oncorhynchus tshawytscha*).

Administration of steroid by incorporation in feed has a long history of use (for reviews see Schreck, 1974 and Hunter and Donaldson, 1983). Steroid is dissolved in a carrier (*e.g.*, ethanol or acetone), uniformly mixed with feed, and allowed to dry before use. Tilapia fry are fed for 21 to 35 days, beginning somewhere between 10 and 14 DPF. Although this technique results in successful sex inversion, certain inefficiencies are cause for concern. The dose received by an individual fish is likely variable, due to differences in body size and social status. The culturist must then accept partial or incomplete sex inversion or increase the treatment dose to beyond the optimal requirement to achieve 100% sex inversion. Furthermore, the long period of treatment employed by typical feeding methods results in human handling of anabolic steroid three to five times daily for up to 35 days. This degree of handling presents an added risk to the aquaculture worker, given the tumorigenic and teratogenic effects of anabolic androgenic steroids (Lewis and Sweet, 1993). This risk is easily mitigated by the establishment of proper handling procedures. However, often these precautions are not properly implemented. For instance, in developing countries where much of the worldwide tilapia production occurs, disposable rubber gloves for the handling of treated feed may be either unavailable or too expensive to be practical. Furthermore, it is common in developing countries for workers with little or no protective clothing (*e.g.*, rubber waders) to wade in ponds containing dissolved steroid while grading or sampling treated fish. Therefore, techniques that minimize worker exposure to anabolic steroid, but are as (or more) effective as feeding treatments need to be established.

Torrans et al., (1988) described an immersion technique for masculinization of blue tilapia, using the synthetic androgen MB. Steroid solutions were exchanged weekly over the five-week treatment period; thus, steroids were handled directly only five times. However, the total treatment period remains similar to feeding methods and requires that fry be held in tanks during treatment. The technique described in our study consisting of immersion in MDHT decreases the treatment period, thereby reducing worker exposure while still achieving nearly complete masculinization.

Table 2. Average (±SE) weight, total length, and sample size (n) from experiment two. Data collected after sampling (100 DPF) for determination of sex distribution. Group abbreviations are the same as given in Fig. 1.

Group	Average Weight (g)	Average Length (mm)	n
MT-100	2.77±0.21	41.7±1.2	29
MT-500	3.45±0.21	44.4±1.0	30
MDHT-100	2.95±0.30	41.7±1.4	29
MDHT-500	3.29±0.22	43.7±1.1	33
ETH	2.97±0.26	42.5±1.3	29
CTL	3.17±0.23	42.2±1.0	50

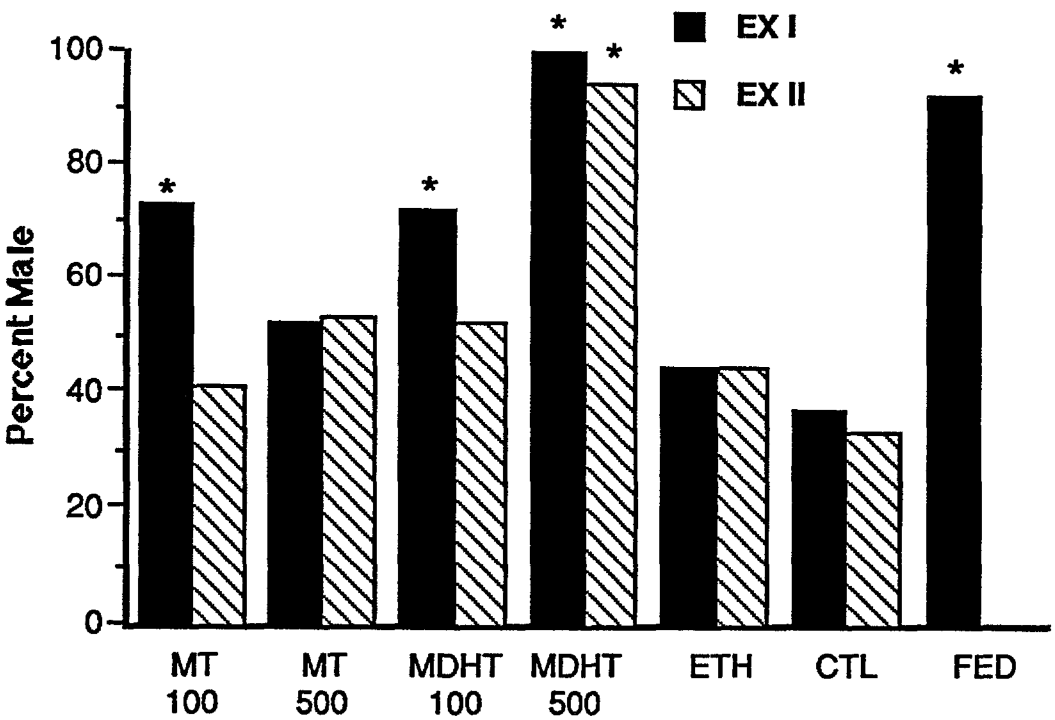

Figure 1. Percent males in each group for experiments one (EX I) and two (EX II). Group designations are as follows: immersion treatment in 100 or 500 µg 17α-methyltestosterone/l (MT 100, MT 500), immersion in 100 or 500µg 17α-methyldihydrotestosterone/l (MDHT 100, MDHT 500), immersion in ethanol vehicle (ETH), immersion in water alone (CTL), and methyltestosterone feeding treatment (FED) from 10-30 DPF (60 mg/kg feed). Asterisks indicate significant (from chi square test; a ≤ 0.05) differences in proportion of males from the pooled control (ETH and CTL) group. Sample sizes are given in Table 1.

Masculinization of Nile tilapia by immersion in MDHT may provide a practical alternative to the use of steroid-treated feed. Shortening the period of treatment and amount of handling required reduces the risk of worker exposure to anabolic steroids. Furthermore, fish are confined for only three days during the treatment procedure, and our results suggest that MDHT immersion does not affect mortality or growth. This technique shows much promise, but further evaluation is needed before application in large scale aquaculture operations.

Anticipated Benefits

Immersion in 17α-Methyldihydrotestosterone may provide an alternative method of masculinization to dietary steroid treatment. Immersion provides a substantially reduced treatment period and increased control of worker and environmental exposure to anabolic steroids. Furthermore, fry exposure to steroid may be more uniform and more efficient using immersion than feeding techniques.

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Maximum Voluntary Feed Intake and Growth of Nile Tilapia Fry as a Function of Water Temperature

Work Plan 7, Africa Study 4

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Introduction

Production of monosex tilapia fingerlings is often the primary constraint to broad adoption of food fish production strategies evaluated through the PD/A CRSP. Perhaps the most widely used method for production of monosex fingerlings is masculinization of fry by the use of hormone-treated feed (sex reversal). In recent years much PD/A CRSP research has focused on this process.

Influence of water temperature on dynamics of tilapia growth has implications for many aspects of tilapia culture, including sex reversal. Growth rate has long been suspected to influence timing of gonadal differentiation and efficacy of sex reversal, but little research data is currently available. However, before these investigations can be conducted, more information is needed on appetite and growth response of tilapia fry grown at temperatures commonly found in sex-reverse culture situations. Additionally, information on maximum feed consumption of fry as a function of temperature is necessary to most efficiently administer the hormone-treated feed. Given growing consumer concern about food safety, the ability to eliminate excess use of hormone is highly desirable.

Maximum voluntary feed intake (satiation) of fry fed a powdered feed cannot be measured using standard techniques. For this study, therefore, maximum voluntary feed intake was defined as the feed rate which growth no longer increased. The desired end result of this study is a set of tables, by temperature, listing satiation feeding rates in percent body weight per day (% bw/d) for fish weighing 15 mg, 25 mg, 50 mg, 75 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, or 900 mg.

Materials and Methods

This experiment was conducted at the Auburn University Fisheries Research Unit in Auburn, Alabama. One trial was run at each of the following three temperatures: 30°C, 26°C and 22°C. The 30°C and 26°C trials were continued for a period of 28 days, while the 22°C trial lasted 32 days. For each trial, 300 *Oreochromis niloticus* (Egypt strain) fry weighing 16-26 mg (10.7-11.8 mm average length) were stocked into each of 28 45-l aquaria. Fry were counted individually and weighed in groups of 300 to determine average weight. Treatments of four replicates were fed at seven feed rates, ranging from 10-28% bw/d at 30°C, 7-25% bw/d at 26°C and 4-22% bw/d at 22°C (Table 1). Fish were fed Zeigler's tilapia starter control diet (54% protein). Daily feed rations, measured to the nearest 0.01 g, were divided into four approximately equal portions and fish were fed at equally spaced intervals during daylight hours, except on sampling days, when the daily ration was divided into three feedings. Feed rates were adjusted daily, based on actual aquarium population (as counted at each sampling) and anticipated growth rate (assumed to be the same as that exhibited during the previous four-day period). Fish were sampled every four days by counting all fish in each aquarium and weighing them as a group to determine total biomass. Total weight, number of fish and growth over the previous four days were used to determine feed rate for the next four days, which was adjusted daily. Temperatures were checked, and adjusted if necessary, at least four times daily. At all times, temperature remained within 1°C of the desired temperature. Water flow was maintained at a minimum of two exchanges per hour. All aquaria were siphoned regularly to remove uneaten feed and accumulated debris. Dissolved oxygen (DO), total ammonia nitrogen (TAN) and pH were measured regularly at the outfall from the aquaria. Total alkalinity and total hardness were measured at the beginning and end of each trial.

30° Trial 28 days Initial size = 16.1 mg			26° Trial 28 days Inital size = 17.5 mg			22° Trial 32 days Initial size = 25.9 mg		
Feed rate (% bw/day)	Avg. final weight (mg)	Growth (mg/d)	Feed rate (% bw/day)	Avg. final weight (mg)	Growth (mg/d)	Feed rate (% bw/day)	Avg. final weight (mg)	Growth (mg/d)
10	428	14.7	7	179	5.6	4	92	2.1
13	565	19.6	10	285	9.5	7	167	4.4
16	685	23.9	13	366	12.4	10	228	6.3
19	730	25.5	16	421	14.4	13	265	7.5
22	785	27.5	19	463	15.9	16	264	7.4
25	833	29.2	22	496	17.1	19	306	8.8
28	900	31.6	25	531	18.3	22	314	9.0

Table 1. Feed rates, average final weights, and growth of Nile tilapia fry reared for 28-32 days at 30°C, 26°C and 22°C.

Data were analyzed by regression and curve-fitting techniques. A regression equation was determined for fish growth in each aquarium. These equations were then used to find growth rates of the fish in those aquaria at specific sizes. The resulting points were plotted against feed rate to show, for fish of a given size at each temperature, at what feed rate maximum growth occurred. This feed rate would then be considered maximum voluntary feed intake.

Results

Average final weights and growth of fry at each temperature are shown in Table 1. Upon analysis of the data, it became apparent that the highest feed rates used at each temperature did not exceed maximum voluntary feed intake for the smallest sizes of fish. Additionally, fish at the lower feed rates did not reach a size sufficient for adequate comparison with fish fed at the higher rates. Consequently, the data collected so far is insufficient to determine maximum feed consumption for fish at all sizes encountered during the first 28 days of feeding at these temperatures. Further trials are planned to obtain the needed data points.

Anticipated Benefits

Results from this study will contribute to an understanding of appetite and growth response of tilapia fry reared at a wide range of temperatures. This information will enable researchers to investigate the effects of growth rate on timing of gonadal differentiation and the efficacy of sex reversal. It is anticipated that these results will lead to the more efficient use of hormone-treated feed and will help lead to the clearance of 17–α methyltestosterone for this purpose by the U.S. Food and Drug Administration. Tilapia farmers worldwide, and especially those in the United States, will benefit greatly from the clearance of this compound for commercial use.

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Characterization of African Soils and Site Evaluation

Work Plan 7, Africa Study C

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Introduction

On-site CRSP research in Rwanda ended in April, 1994 as a result of war and civil violence following the deaths of the presidents of Rwanda and Burundi. Continued lack of security has prompted the relocation of research to the U.S. along with initiation of a search for a replacement African site. Objectives of this study included the development of site selection criteria, collection of data and evaluation of promising sites, and characterization of African soils. Site evaluation continues in the Interim Work Plan, Study 1, a cooperative work plan with the Africa/ Auburn PD/A CRSP.

Materials and Methods

Fifteen site evaluation criteria for prime and companion sites were developed with input from the Technical Committee and ME. USAID requirements for new CRSP sites were attached. Information on potential sites was solicited from the CRSP and gathered from other sources. AID planning documents for all Sub-Saharan African countries were examined to determine current AID program status and strategic objectives. Promising countries and sites were identified and requests to travel to the two most promising sites initiated. Soils were collected from two African sites using a 5-cm core forced 15 cm into the substrate. At least three cores were taken from four locations at two sites. Soils were characterized for CEC, sand, silt and clay content, pH, lime requirement, exchangeable bases, and base saturation.

Results

Promising sites were identified at Sagana, Kenya; Malawi (Bunda College station and Domasi Experimental Fish Farm) and several sites in Zimbabwe. Travel approval was requested for Malawi and Kenya, the most promising sites. Travel to Malawi was not approved by AID Malawi; the trip to Kenya was completed November, 1994 by Seim and Egna. Sagana Fish Culture Farm, about 100 km northeast of Nairobi was found to have the potential to meet most selection criteria for a new site, but support from the Kenya Department of Fisheries was not encouraging. A Kenya policy of one international project per site made location there uncertain in light of the current Belgian studies at Sagana. [New leadership in the Dept. of Fisheries is now supportive of CRSP presence at Sagana. Deputy Director (and acting Director) of Fisheries Oduor would be supportive of a direct agreement with the CRSP should that site be selected]. Information on other potential sites in Africa was also received.

Five composite soil samples were brought back from Kenya in November, 1994. Four of the composite samples came from the Sagana Farm and one from the Baobab Farm near Mombasa. Of the five, three proved to be quite alkaline and two quite acid. All samples from Kenya had very high clay contents (49.7 - 81.7%). Sagana soil samples were low in pH for a pond not recently in use and near neutrality for ponds more recently in use. Previously collected Rwandan soils were combined to form six composite samples (Table 1). All samples from Rwanda were very acid (pH 3.9-5.6), and base saturation levels were generally low. One of the Rwandan composite soils was very high in organic matter (OM); this high OM level was reflected in the CEC of this soil (62.6 meq/100g), and, in the extremely high lime requirement estimates obtained by all methods used.

Discussion

Further investigation of Sagana appeared warranted. That station is quite large, and some 40 ponds with a total water area of about 15 ha now exist, with additional land area and water capacity in reserve. There are no restrictions on use of fish species of interest but the capacity to conduct CRSP chemical analyses is limited and would have to be expanded. The Africa project lost essentially all its equipment and supplies, destroyed or left behind in Rwanda. Much of this would have to be replaced before a new site would be operational. This would be especially true for Sagana Station which currently has limited analytical capacity. Malawi is still considered of interest because of a strong desire by Malawians at Bunda College for collaboration, and the importance of aquaculture in Malawi. The presence of ALCOM (Aquaculture for Local Community Development of FAO) in Malawi gives it potential as a regional center for aquaculture. Restrictions on the use of *Oreochromis niloticus*, however, may allow only companion site consideration for Malawi. Regardless, links with ALCOM and Malawian aquaculturists would broaden networking and information exchange from any African site. Zimbabwe, Zambia and Tanzania remained under consideration.

Strategic Objectives as published were found less informative than Mission interpretation and application. "Food Security" for instance, would not necessarily include aquaculture as being within the Mission objectives. Site evaluation and development planning continue under the Interim Work Plan, Study 1.

Anticipated Benefits

The selected site will be chosen both for local and regional impact in Africa. Countries south of Kenya are organized within SADC (Southern African Development Community). Ties with SADC will be sought regardless of the prime site location to extend the regional impact of the CRSP activities in Africa. An apparent need in Africa is the development of efficient and sustainable strategies for pond aquaculture, and the training of individuals to extend this information to the farmers. CRSP objectives under the new proposal would contribute to meeting this need. Planning to emphasize regional impact takes advantage of existing networks and encourages the development of links between aquaculture scientists working in Africa to expand impacts.

Reproductive Efficiency of Nile Tilapia *(Oreochromis niloticus)* and "Red" Tilapia *(Oreochromis spp.)* and Comparative Growth and Efficacy of Sex Reversal of Nile Tilapia and "Red" Tilapia

Interim Work Plan, Africa Study 6

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Introduction

Tilapia are cultivated in Africa, southern Asia and Central and South America. The "red" tilapia *(Oreochromis spp.)* has greater acceptance among some consumers, but "normal" colored fish showed superior growth compared to red colored fish. This research compares Nile tilapia and "Red" tilapia as relates to the reproductive efficiency of adults and growth, survival, and response to sex reversal of fry.

Sex reversal involves feeding a feed that has been treated with a male hormone to fry before the primal gonadal cells of females have differentiated into ovarian tissue. The hormone-treated feed used for sex reversal is generally stored under refrigeration. Incorporated in this research is an analysis of the shelf life at ambient tropical temperatures of the hormone-treated feed used for sex reversal. The following is a preliminary report summarizing methods and results without statistical analysis. All research was conducted at "El Carao" Aquaculture Station in Comayagua, Honduras.

Materials and Methods

"Red" tilapia brood fish were compared to *O. niloticus*, ("Black") in their ability to produce fry for sex reversal. Four reproductive trials of were conducted for each species. *O. niloticus* or "red" tilapia broodfish were stocked at a rate of 230 females and 115 males into 0.05 ha ponds (882-1,554 kg/ha). Ponds had been filled with reservoir water 2 to 3 days prior to stocking. Two ponds were stocked with red broodfish and two ponds with Black broodfish. The mouth of each female was checked for eggs or fry at stocking. Fish were fed once daily at a rate of 1% body weight per day. Maximum and minimum water temperature, and morning dissolved oxygen (D.O.) were recorded daily and secchi disk visibility weekly. Ponds were drained and fry collected 215 to 230 degree days after stocking (13-19 days) as suggested by Green and Teichert-Coddington (1993). Fry were collected by draining ponds to a concrete harvest sump. All fry were then graded through 3.2 mm vexar screen (Hiott and Phelps 1993) and were counted as two separate groups: those retained by the grader (>14 mm) and those passed through the grader (<14 mm). Subsamples of 25 to 50 fry were measured to the nearest millimeter at the beginning, middle and end of counting. All fry were counted by visual comparison. All broodfish were separated by sex, counted, and weighed. They were placed in 20 m^3 concrete tanks and fed at 1.5% body weight per day for 7 to 10 days prior to restocking. Each pond was then thoroughly dried and prepared for refilling. The above procedure was replicated four times. Fry from these trials were used for sex reversal studies.

Fry of Nile and "Red" tilapia < 14 mm were stocked in hapas suspended from a wooden pier in a 0.1-ha pond with a maximum depth of 1.2 m and a minimum depth of 0.7 m. The hapas measured 1.0 x 1.0 x 0.7 m (length x width x height) and contained 0.5 m^3 of water. Fry were counted by visual comparison and stocked at 4,000 m^3.

Fry were fed four times daily for 28 days. The feeding rate was adjusted weekly by measuring 25 fish to the nearest millimeter and estimating weight as described by Shelton et al. (1978). Daily feeding rates were 15%, 12%, 8%, and 4 % BW during weeks 1, 2, 3, and 4, respectively. Daily feed quantities were weighed and placed in sealed plastic jars until delivery. A feed containing 60 mg/kg of 17-α methyltestosterone was prepared by following the method described by Zeigler Inc. (personal communication) and the protocol presented in INAD #.

The hormone feed was prepared by dissolving three g of the steroid in 1,000 ml of 95% ethyl alcohol to make a stock solution with 3 mg MT/ml. Twenty milliliters of the stock solution were mixed with 210 ml of 90% ethyl alcohol and sprayed on one kg of the feed as it was mixed for 20 min in a covered industrial mixer. The feed was then spread out for 12 hours to evaporate the solvent. The next day the feed was sealed in plastic zip-lock bags and placed in a freezer at -2°C.

Preliminary Results

Preliminary results are summarized in tables 1 and 2.

Table 1. Summary of reproductive efficacy trials with "Red" and Nile Tilapia. Each entry is the average of two replicates.

Trial	Total No. of Fry	Total No. of Sex-reversible fry <= 14 mm	Tot. No. of Large Fry > 14 mm	Degree Days to Harvest	Avg. Temp °C	No.Females	No. Males	No. Fry/kg Female
Trial 1 Blacks	37329	36388	316	218.0	25.3	220.0	108	1440
Trial 1 Reds	35329	33388	816	221.3	25.3	213.0	103	1230
Trial 2 Blacks	60500	57250	750	228.0	29.9	221.0	104	2427
Trial 2 Reds	58700	54500	750	225.0	29.9	228.0	107	2362
Trial 3 Reds	96050	92000	2500	231.5	29.5	224.0	114	3506
Trial 3 Blacks	76000	74750	1150	230.5	29.5	227.0	114	3048
Trial 4 Reds	114250	110750	1500	226.3	31.2	0.0	0	3732
Trial 4 Blacks	118750	116750	500	226.3	31.2	230.0	115	3688
Avg. Blacks	72695	70597	679	225.0	29.0	226.3	111	2635
Avg. Reds	76532	73347	1392	226.8	29.0	164.5	80	2724

Table 2. Comparative growth and efficacy of sex reversal of Nile tilapia (Bk) and "Red" tilapia (Rd. Treatment refers to number of days treated with 60 mg/kg MT for "Red" tilapia or "Black" *O. niloticus*.

Treatment	Final Length (mm)	Growth (mm/d)	Wt. Harvested (g)	Avg. Weight (g)	% Survival	FCR
14-d Rd	20.0	0.7	335.7	0.18	95.6	0.6
14-d Bk	22.1	0.9	363.1	0.22	83.9	0.5
21-d Rd	27.0	0.8	537.9	0.34	79.3	0.8
21-d Bk	27.1	0.8	591.4	0.38	78.1	0.7
28-d Rd	31.6	0.8	784.6	0.56	70.1	0.8
28-d Bk	32.4	0.8	952.9	0.64	74.7	0.9
Cont. Rd	32.6	0.8	877.4	0.64	68.5	0.9
Cont. Bk	33.0	0.8	1044.8	0.65	80.5	0.8

Growth and Efficiency of Sex Reversal of Nile Tilapia Fed Hormone Treated Feed Stored Under Different Storage Regimes

Interim Work Plan, Africa Study 7

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Introduction

Tilapia are cultivated in Africa, southern Asia and Central and South America. The "red" tilapia (*Oreochromis spp.)* has greater acceptance among some consumers, but "normal" colored fish showed superior growth compared to red colored fish. This research compares Nile tilapia and "red" tilapia as relates to the reproductive efficiency of adults and growth, survival, and response to sex reversal of fry.

Sex reversal involves feeding a feed that has been treated with a male hormone to fry before the primal gonadal cells of females have differentiated into ovarian tissue. The hormone-treated feed used for sex reversal is generally stored under refrigeration. Incorporated in this research is an analysis of the shelf life at ambient tropical temperatures of the hormone-treated feed used for sex reversal. The following is a preliminary report summarizing methods and results without statistical analysis. All research was conducted at "El Carao" Aquaculture Station in Comayagua, Honduras.

Materials and Methods

Experimental design and procedure were conducted in the same manner as the above study except for the following. Feeds for this experiment were taken out of the freezer at their designated times and placed in a refrigerator at 4°C or "on the shelf" at tropical ambient temperature (28°C +/- 1.5°C). Feed storage times were: a) zero months in the refrigerator - 26 days on the shelf, b) zero months in the refrigerator - seven days on the shelf, c) zero months in the refrigerator - zero days on the shelf, d) two months in the refrigerator - 26 days on the shelf, e) two months in the refrigerator - seven days on the shelf, and f) two months in the refrigerator - zero days on the shelf. Fry were fed daily for 14, 21, or 28 days. These studies began on February 7 and finished on August 31.

Preliminary Results

Preliminary results are summarized in Table 1.

Table 1. Summary of the growth and efficiency of sex reversal of Nile tilapia fed hormone-treated feed stored under different storage regimes.

Treatment Refrig.-Shelf	Avg. Length (mm)	Total Wt. (g)	Avg. Wt. (g)	Total Number	Avg. % Survival	FCR
Omo-Oday	36.1	964.4	0.87	1106	55.3	.94
Omo-7day	36.0	1038.8	0.90	1154	57.7	.88
Omo-28day	36.0	1081.5	0.87	1250	62.5	.85
2mo-Oday	35.0	970.4	0.79	1223	61.1	.95
2mo-7day	36.4	990.4	0.87	1141	57.0	.94
2mo-28day	36.1	975.4	0.90	1083	54.2	.96
Control	37.0	1182.6	0.99	1189	59.5	.79

Southeast Asia

Researchers at the Asian Institute of Technology in Thailand undertook a study to evaluate caging densities and pond loading rates for tilapia that were caged and fed within semi-intensive ponds with small tilapia at large. Such a system could be an effective means to produce large tilapia efficiently. Caged tilapia were stocked at five densities, and held in ponds loaded at two rates for 90 days of culture. Growth rates of the caged tilapia were similar regardless of stocking density; however, survival rates differed significantly with cage density, with fish at higher densities exhibiting very high mortality rates. Growth and mortality rates of the uncaged tilapia were similar to rates found using other culture systems, even though the only source of nutrients was the unused feed and excretory products of the caged fish. Water quality did not deteriorate within the ponds at either loading rate. Cage stocking densities of 64 fish per m^3 resulted in good survival and significant growth.

Increasing the carrying capacity of the pond or size at harvest of tilapia requires more intensive management, which largely involves supplemental feeding. Researchers attempted to determine the upper limits to tilapia production using supplemental feeds. Fish were stocked at four densities, and fed to satiation during the 146-day culture period. The highest growth rate and survival occurred in lower-density ponds. Researchers could not explain why the higher densities did not have correspondingly high growth and survival rates. The best recommendation currently is to stock fish at $3/m^2$ under intensive feeding regimes.

Researchers investigated whether adding carp to a tilapia monoculture would increase the productivity of the pond system. Because tilapia are primarily planktivores, researchers hypothesized that the addition of carp would increase productivity by converting currently unutilized benthic matter into fish flesh. Researchers stocked ponds with tilapia and added carp at various stocking rates. Ponds were fertilized weekly with chicken manure, urea, and triple sodium phosphate (TSP). Preliminary results indicate slow, uniform growth for tilapia, possibly because larger tilapia than called for in the experimental protocol were stocked erroneously. Carp growth proved to be extremely sensitive to and inversely related to stocking density. Although turbidity was higher in ponds stocked with carp, there was little indication of any other difference in water quality between the monoculture and the polyculture ponds.

Researchers at University of Hawaii investigated carbon dioxide (CO_2) exchange between pond water and the atmosphere. Although oxygen exchange is routinely estimated in free water studies, far less attention has been given to diffusion of carbon dioxide, which may be significant. Researchers analyzed data from their pond research facility in the U.S. to quantify the rates of exchange of carbon dioxide between pond water and the atmosphere in fertile earthen ponds, and to identify factors which determine these rates of exchange. An analysis of these data showed that total carbon dioxide concentrations varied little during the day, but showed a perceptible dip during midday, reflecting photosynthetic uptake. Wind speeds directly above the water surface were measured, and researchers observed that the windiest periods occurred mainly during daylight hours. Analysis showed that the concentration of free carbon dioxide and wind speed together accounted for 81% of the variation in the diffusion rates during the diel cycle. Thus, prediction of diffusion rates requires only observed carbon dioxide concentrations and wind speed, although photosynthetic demand can be the primary determinant of concentrations under some conditions.

Deep (approximately 2.5 m), rain-fed ponds are more severely density stratified than the commonly used shallow ponds, and are, therefore, less often stirred by convective overturn at night or by wind-induced mixing. This makes oxygen depletion in the hypolimnion more likely. A study was conducted to describe and quantify the diel temperature cycles and dissolved oxygen (DO) stratification in these deep ponds. During sunny, dry season days, the pond had a slightly deeper mixed layer (at least 35 but much less than 75 cm) than is characteristic of ponds at the more sheltered Asian Institute of Technology site. The bottom water below 2 m depth was almost completely isolated from the upper water, receiving only minimal transport of oxygen from above. During the rainy season the isolation below 2 m was maintained even through a dark rainy day. These results show that active mixing may be necessary to maintain deep ponds as suitable culture environments for some animal species.

A Finishing System for Large Tilapia

Work Plan 7, Study 3

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Introduction

Pond carrying capacity is largely determined by management practices. Earlier work on semi-intensive culture of Nile tilapia *(Oreochromis niloticus)* using manure or inorganic fertilizers indicated that carrying capacity might reach 2,000 to 3,000 kg/ha (Diana et al. 1991a, b; Knud-Hansen et al. 1991). As stocking density is increased in fertilized ponds, carrying capacity remains largely the same and density-dependent growth occurs (Diana et al. 1991b). Thus, the ultimate size of fish at harvest is largely related to density stocked in fertilized ponds, while biomass at harvest is more consistent regardless of stocking density. Maximum size at harvest for these fertilized ponds is approximately 250 g for fish grown five months.

Increasing the size at harvest of tilapia requires more intensive management, which largely involves supplemental feeding. CRSP experiments with supplemental feeding indicated that tilapia can reach 500 g in 5 months when feed and fertilizer are provided in combination (Diana et al. 1994, 1995). Such experiments were done at fish densities of 3 fish per m^2, which would cause density-related declines in growth for fish in fertilized ponds. However, the addition of supplemental feed increased the growth rate of fish stocked at high density, and resulted in a higher carrying capacity for the pond. The limit on such feeding and density increases would occur when conditions in the ponds reach limiting levels due to increased oxygen demand, build up of metabolites, or other factors which produce poor water quality. Such a limit to tilapia production was demonstrated for Honduran ponds at 3 fish/m^2 (Green 1992), while Diana et al. (1994) found no decline in water quality for tilapia stocked at 3 fish/m^2 in Thai ponds, and Diana and Lin (1996) found no decline in water quality even up to 9 fish/m^2. In the latter studies, concomitant fertilization probably helped maintain reasonable water quality.

Another possible system to grow large tilapia under intensive culture would be to grow tilapia in submerged cages within tilapia ponds. In such a system, waste feed, feces, and excretory products of the caged tilapia would increase nutrient levels for the tilapia at large in the pond, possibly resulting in their rapid growth as well. The limits to such a system would be how many fish caged fish a pond could support, and how many fish could be supported in each cage. The purpose of this experiment was to determine if tilapia could be grown to large sizes in cages submersed within ponds used for tilapia culture. This is a logical extension of earlier work on polyculture of *Clarias* in cages with tilapia in ponds (Lin and Diana, 1995), and would allow both a finishing system and grow out system with limited infrastructure.

The objective of this study was to evaluate caging densities and pond loading rates for tilapia caged and fed intensively in ponds with smaller

Table 1. The biomass, number, and mean size of tilapia stocked and harvested from each pond and cage.

Pond	Cage	At Stocking			At Harvest		
		Number	Biomass	Mean Size	Number	Biomass	Mean Size
L1		626	1.6	2.6	496	87.0	175.4
	L16	16	6.4	400.0	17	15.4	905.9
	L16	16	6.4	400.0	16	13.9	868.8
	L32	32	11.4	356.3	23	19.5	847.8
	L32	32	11.7	365.6	20	16.0	800.0
	L64	64	22.0	343.8	48	40.4	841.7
	L64	64	22.3	348.4	11	7.7	700.0
L2		626	1.8	2.9	522	57.8	110.7
	L16	16	5.1	318.8	7	3.9	557.1
	L16	16	5.3	331.3	5	3.2	640.0
	L32	32	11.7	365.6	2	1.4	700.0
	L32	32	11.5	359.4	3	1.8	600.0
	L64	64	22.3	348.4	19	13.1	689.5
	L64	64	22.2	346.9	3	1.8	600.0
L3		626	1.7	2.7	543	79.1	145.7
	L16	16	6.0	375.0	15	11.1	740.0
	L16	16	7.1	443.8	16	11.7	731.3
	L32	32	10.3	321.9	30	20.0	666.7
	L32	32	11.1	346.9	30	19.4	646.7
	L64	64	22.5	351.6	26	16.6	638.5
	L64	64	23.3	364.1	45	29.3	651.1
H1		626	1.8	2.9	530	80.4	151.7
	H16	16	6.5	406.3	9	5.2	577.8
	H32	32	11.3	353.1	3	1.5	500.0
	H64	64	20.0	312.5	43	25.4	590.7
	H64	64	21.7	339.1	24	12.0	500.0
	H128	128	41.6	325.0	29	16.0	551.7
	H256	256	88.7	346.5	63	32.4	514.3
H2		626	1.6	2.6	578	97.6	168.9
	H16	16	5.9	368.8	15	11.7	780.0
	H32	32	11.4	356.3	13	8.9	684.6
	H64	64	22.5	351.6	63	56.1	890.5
	H64	64	21.9	342.2	20	13.5	675.0
	H128	128	45.1	352.3	48	37.7	785.4
	H256	256	87.4	341.4	34	25.9	761.8
H3		626	1.8	2.9	559	84.6	151.3
	H16	16	6.8	425.0	6	4.4	733.3
	H32	32	10.5	328.1	8	4.9	612.5
	H64	64	22.8	356.3	11	7.0	636.4
	H64	64	21.5	335.9	7	4.0	571.4
	H128	128	43.0	335.9	33	19.3	584.8
	H256	256	85.6	334.4	23	15.8	687.0

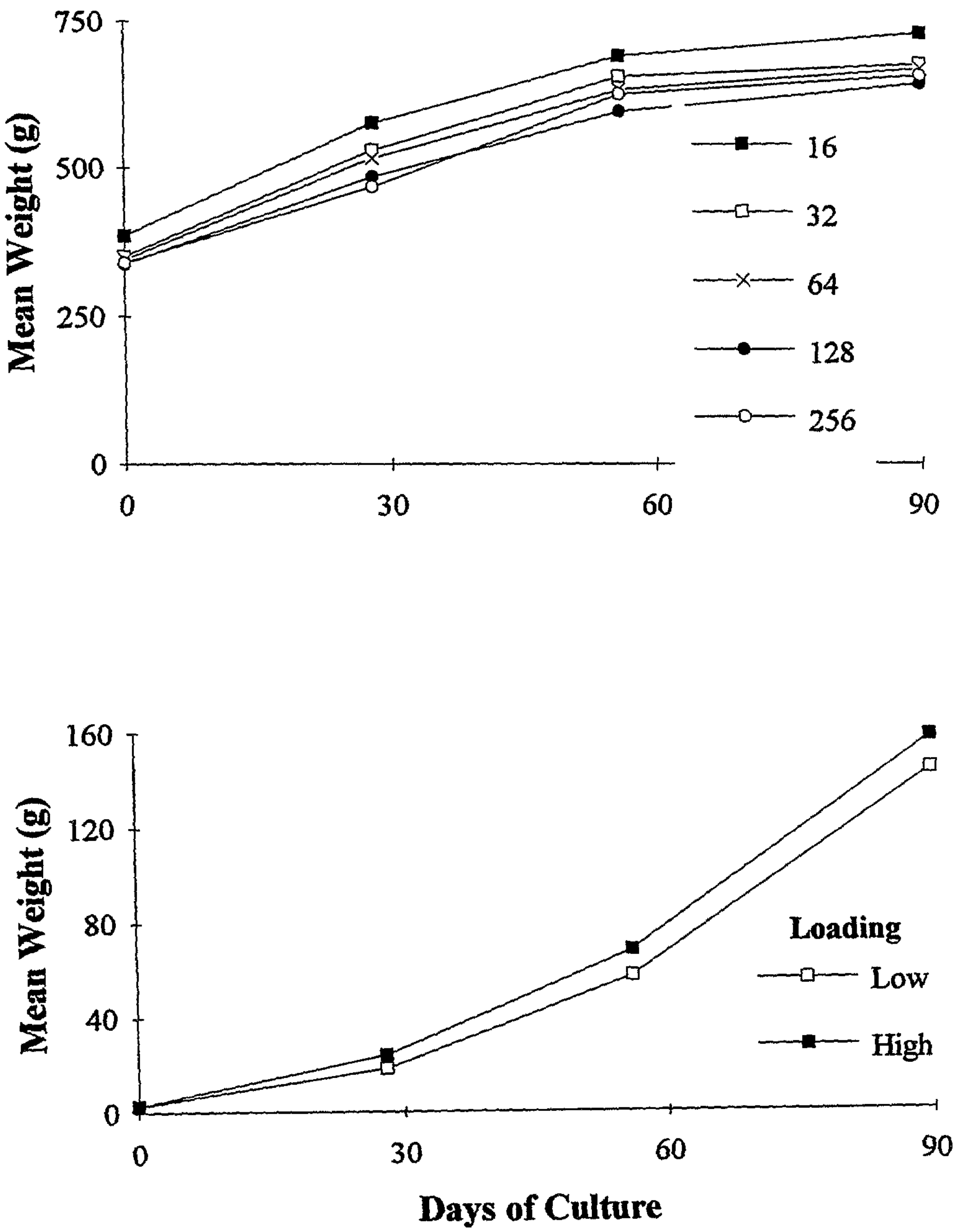

Figure 1. Changes in mean weight during culture for tilapia in cages at different densities (upper) and in ponds with different cage loading rates (lower).

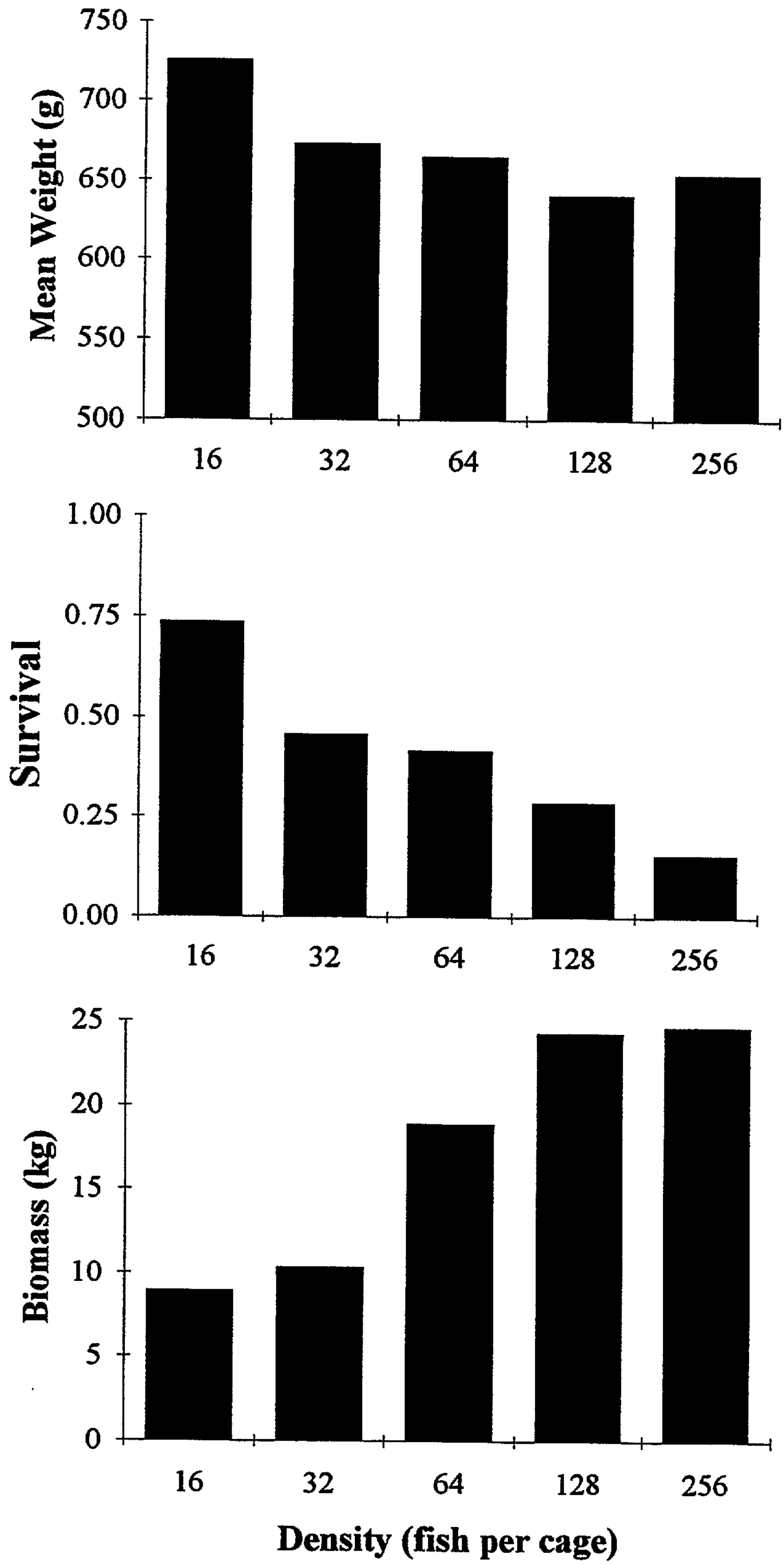

Figure 2. Final weight (upper), survival (middle), and biomass (lower) at each density for fish in cages at different densities.

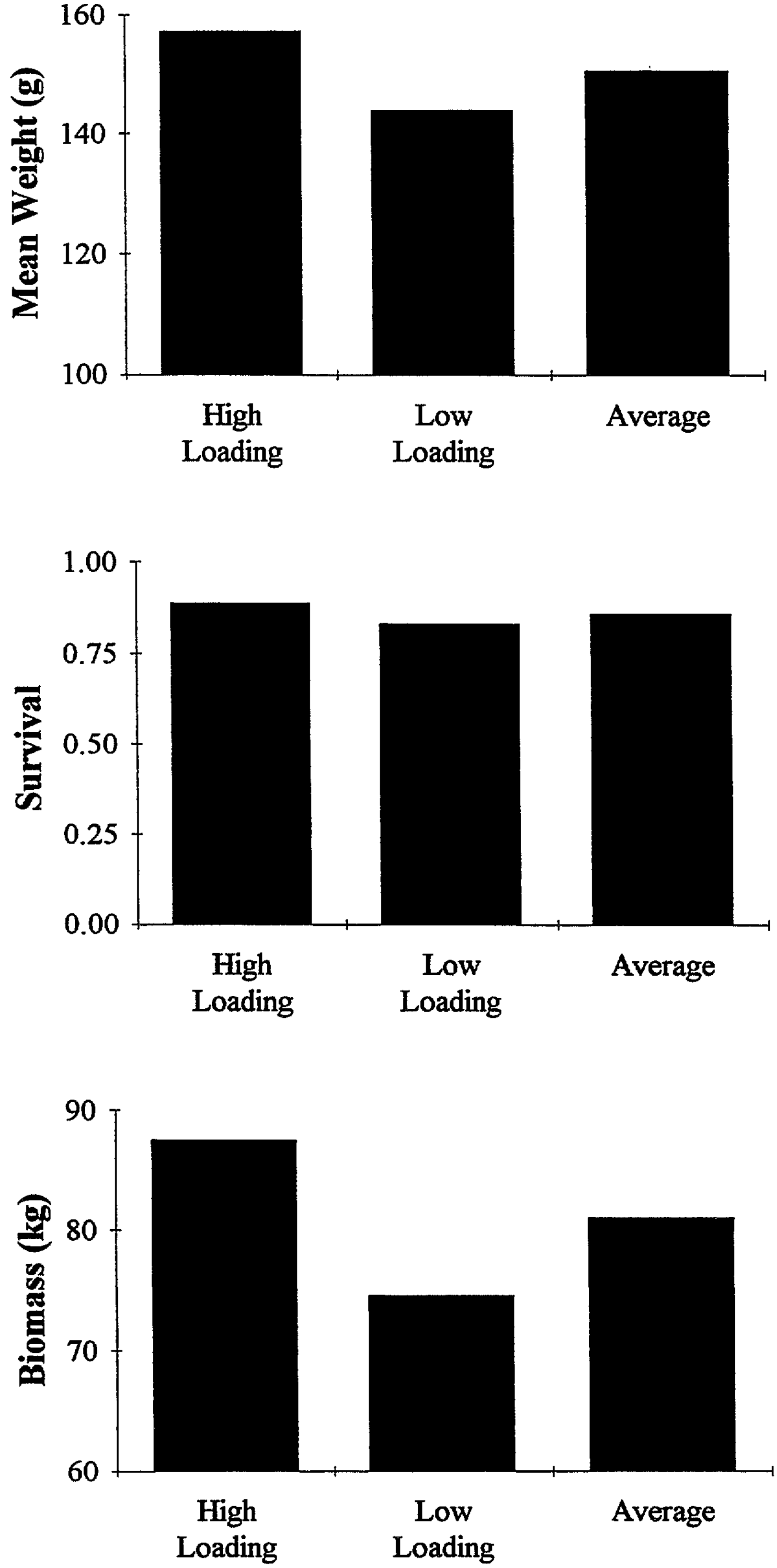

Figure 3. Final weight (upper), survival (middle), and biomass (lower) at each density for fish in ponds with different cage loading rates.

tilapia at large. This was tested by a two dimensional test: cages with different fish densities (16, 32, 64, 128, and 256 fish per m^3) and ponds with different loading rates of caged fish (224 and 560 caged fish per pond).

Materials and Methods

Data for this study were collected from 6 ponds at the Asian Institute of Technology, northwest of Bangkok, Thailand. The ponds used in the experiment were 313 m^2 in surface area and normally filled to 1 m. Ponds were stocked with 2 fish/m^2 on 22 September 1993, with fish averaging 2.7 g. Prior to pond stocking, cages (1 m^3 in volume) were submersed into each pond and stocked with large tilapia at different densities. Cage densities were 16, 32, 64, 128, and 256 fish per cage, with fish averaging 346 g at stocking on 15 September 1993. Ponds with low loading rates received 6 cages, 2 each at densities of 16, 32, and 64 fish (224 caged fish per pond). Ponds with high loading rates received 6 cages, with 1 cage each at densities of 16, 32, 128, and 256 fish per cage and 2 cages at 64 fish per cage (560 caged fish per pond). Fish in cages were fed to satiation daily, with satiation determined on a weekly basis using floating feed. The average satiation rates for cages at a given density were subsequently applied to each cage for the remainder of the week. In addition to feeding, ponds were not fertilized.

Physical and chemical data were collected in a similar manner to earlier experiments (Diana et al. 1991a, 1994). Water quality data were collected biweekly. Meteorological data, including solar radiation, rainfall, and wind speed were collected daily. Vertical distribution of dissolved oxygen, temperature, pH, alkalinity, and ammonia was determined at 0600 hr, 0900 hr, 1400 hr, 1600 hr, 1800 hr, 2300 hr, and 0600 hr in each pond. These diurnal analyses were repeated biweekly on water from the top (25 cm), middle, and 25 cm above the bottom of the water column. Temperature and oxygen differentials were calculated as the difference between top and bottom measurements at 1600 hr.

Fish in ponds were sampled monthly for average weight. At least 40 fish were seined from each pond and individually weighed. Fish in cages were counted and batch weighed at each sampling.

Statistical analyses were conducted using SYSTAT (Wilkinson 1990). Overall growth (g/day), net yield (kg), and percent survival were calculated for each pond and each cage. Treatment effects on fish or chemical variables were tested with the monthly data set by ANOVA and Tukey's multiple range test. Differences were considered significant at an alpha of 0.05.

Results

Fish weight increased in a linear fashion in cages and ponds throughout the experiment (Figure 1). Overall growth rate did not differ significantly among cage densities nor among fish at large in the two pond treatments (ANOVA, $p < 0.05$, Figure 1, Table 1). Survival of caged fish was not significantly different among pond treatments nor among densities from 16-64 fish per cage (Figure 2). However, higher fish densities did significantly affect survival, with very low fish survival at high fish densities in cages (ANOVA, $P<0.05$, Figure 2). Pond loading rates did not significantly affect growth, survival, or yield of fish at large in the ponds (Figure 3).

Most physical and chemical variables showed no significant differences among pond treatments. Exceptions were chlorophyll *a* and total phosphorus, which were higher in the high density treatments. The high density ponds showed no ill effects of the higher stocking rates and loading rates due to caged tilapia.

Significant mortalities occurred in cages in these experiments, particularly at densities in cages greater than 64 per m^3. Many of these ponds lost more than 80% of their original fish stock (Table 1). While dates of mortalities are available, these cannot be correlated to dissolved oxygen or other characteristics of the ponds at those dates, since the latter data were only collected every two weeks, and many dates with mortalities did not coincide with the dates of water quality collections. Thus, while the water quality overall did not deteriorate in these ponds, it must have reached limiting levels in the cages stocked at high densities.

Discussion

Growth did not differ among cages, indicating that density dependent growth was not a problem in these cages. However, this is a misleading statement because mortality differed dramatically among cages, so the density stocked was not held constant between treatments throughout the experiment. However, within a density of 16-64 fish per cage, there was no density dependent growth or survival.

Growth was rapid in all ponds, and reached rates near usual levels for fertilized ponds. Final size was less than normal dues to the short time period over which the experiments were conducted (90 days), but comparable to growth observed after 90 days of culture (Diana et al. 1991a, 1991b).

The application of this study to tilapia management is fairly obvious. Most rapid growth and highest survival in cages occurred at 16 to 64 fish per cage. The optimal finishing system at present appears to be with tilapia at 64 fish per cage. Water quality did not deteriorate in the high loading rate ponds, at least to the point of killing the tilapia at large in those ponds or in terms of affecting their growth rates. Therefore, it is still unclear how many cages or caged fish could be added to such a pond without water quality deterioration. In fact, since many fish died in the high loading ponds, it is not even clear whether those ponds would have reached deleterious conditions if the fish had remained alive at those caging numbers. Further experiments would be necessary to answer such questions.

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Stocking Density and Supplemental Feeding

Work Plan 6, Study 6

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(Printed as Submitted)

Introduction

Pond carrying capacity is largely determined by management practices. Earlier work on semi-intensive culture of tilapia using manure or inorganic fertilizers indicated that carrying capacity might reach 2,000 to 3,000 kg/ha (Diana et al. 1991a, b; Knud-Hansen et al. 1991). As stocking density is increased in fertilized ponds, carrying capacity remains largely the same and density-dependent growth occurs (Diana et al. 1991b). Thus the ultimate size of fish at harvest is largely related to density stocked in fertilized ponds, while biomass at harvest is more consistent regardless of stocking density. Maximum size at harvest for these fertilized ponds is approximately 250 g for fish grown five months.

Increasing the carrying capacity or size at harvest of tilapia requires more intensive management, which largely involves supplemental feeding. Experiments with supplemental feeding indicated that tilapia can reach 500 g in 5 months when feed and fertilizer are provided in combination (Diana et al. 1994, 1995). Such experiments were done at fish densities of 3 fish per m^2, which would cause density-related declines in growth for fish in fertilized ponds. However, the addition of supplemental feed increased the growth rate of fish stocked at high density, and resulted in a higher carrying capacity for the pond. The limit on such feeding and density increases would occur when conditions in the ponds reach limiting levels due to increased oxygen demand, build up of metabolites or other factors which produce poor water quality. Such a limit to tilapia production was demonstrated for Honduran ponds at 3 fish/m^2 (Green 1992), while Diana et al. (1994) found no decline in water quality for tilapia stocked at 3 fish/m^2 in Thai ponds. In the latter study, concomitant fertilization probably helped maintain reasonable water quality.

The purpose of this experiment was to determine the upper limits to tilapia production utilizing supplemental feeds. In order to test this relationship, fish were stocked at 3, 6, and 9 fish per m^2. These fish were supplementally fed to satiation for 146 days.

Materials and Methods

Data for this study were collected at the Ayutthaya Freshwater Fisheries Station located at Bang Sai (14°45' N, 100°32' E), approximately 60 km northwest of Bangkok, Thailand. The 9 ponds used in the experiment were 280 m^2 in surface area and normally filled to a depth of 1 m. Sex-reversed Nile tilapia *Oreochromis niloticus* averaging 19 g were stocked on 19 October 1994 (Table 1). The ponds were divided into three treatments, with triplicate ponds for each treatment receiving either 3, 6, or 9 fish per m^2 (840, 1680, and 2520 fish per pond). Fish were fed daily to satiation. Feeding rates were readjusted on a weekly basis. Maximum consumption was determined using floating feed, and was estimated individually for each pond. The average consumption for each treatment was then used for the feeding rate over the remainder of the week.

In addition to feeding, ponds were also fertilized weekly to bring a balance of P and N addition to 4 and 1 kg $\cdot$ ha^{-1} $\cdot$ d^{-1}, respectively. This required 1.68 kg urea and 1.0 kg triple super phosphate per week.

Table 1. The biomass, number, and mean size of tilapia stocked and harvested from each pond.

Pond	At Stocking			At Harvest		
	Number	Biomass	Mean Size	Number	Biomass	Mean Size
A1	840	15.4	18	737	307.0	417
A2	840	15.5	18	744	345.5	465
A3	840	15.0	18	745	342.9	460
B1	1680	30.2	18	1355	426.3	315
B2	1680	31.8	19	1248	395.5	317
B3	1680	33.0	20	1103	308.5	280
C1	2520	47.9	19	1471	381.4	259
C2	2520	48.0	19	1782	526.3	295
C3	2520	48.7	19	1723	450.3	261

Physical and chemical data were collected in a similar manner to earlier experiments (Diana et al. 1991a, 1994). Meteorological data, including solar radiation, rainfall, and wind speed were collected daily. For most analyses, combined water samples encompassing the entire water column were taken from walkways extending to the center of the ponds. Pond water analyses, including temperature, dissolved oxygen (both taken at the top, middle, and bottom of the water column), ammonia, nitrate-nitrite, orthophosphate, total phosphorus, alkalinity, pH, Secchi-disk depth, and chlorophyll *a* content were conducted biweekly using standard methods (see APHA 1980 and Egna et al. 1987 for detailed descriptions of methods). Vertical distribution of dissolved oxygen, temperature, pH, alkalinity, and ammonia was determined at 0600 hr, 0900 hr, 1400 hr, 1600 hr, 1800 hr, 2300 hr, and 0600 hr in each pond. These diurnal analyses were repeated biweekly on water from the top (25 cm), middle, and 25 cm above the bottom of the water column. Temperature and oxygen differentials were calculated as the difference between top and bottom measurements at 1600 hr.

Primary production was determined by oxygen changes in the ponds, using methods described by Piedrahita (1988). Daily oxygen production was corrected for diffusion and nocturnal respiration. The overall oxygen production (Gross Primary Production) was then converted to carbon synthesis by relative molecular weights.

Ponds were harvested on 23 March 1995, after 146 days. Final biomass and numbers were determined. Overall growth (g/d) and net yield (kg) were calculated. During the experiment, fish were sampled biweekly for size. About 40 fish were seined from each pond, measured and weighed. Biomass in the pond was estimated biweekly by extrapolating the number of fish in the pond linearly from stocking to harvest, and multiplying this number by the average size of fish.

Statistical analyses were conducted using SYSTAT (Wilkinson 1990). Overall growth (g/day), net yield (kg), and percent survival were calculated for each pond. Feeding rate (%BW/d) was estimated biweekly, while feed conversion rate (FCR) was calculated for overall data and for biweekly data. Average overall values for physical and chemical parameters, total days of culture, and total food input were also calculated. Multiple regressions between growth and density were done to test main effects. Because many of the chemical variables were interrelated, residuals of the above regression were correlated to each physical or chemical variable. Variables which were significantly correlated to the residuals were then examined for auto correlation, and acceptable variables input to the multiple regression to evaluate additional determinants of variations in fish growth, survival or yield. Variables were included in the regression if $p < 0.10$. Treatment effects on fish or chemical variables were tested with the biweekly data set by ANOVA and Tukey's multiple range test. Differences were considered significant at an alpha of 0.05.

Table 2. Growth (g/d), survival (%), yield (kg), feed applied (kg), feed conversion rate (FCR), and forecasted annual yield (kg · ha^{-1} · yr^{-1}) for tilapia from each pond.

Pond	Growth	Survival	Yield	Feed Applied	FCR	Annual Yield
A1	2.71	0.877	291.6	262	0.897	26036
A2	3.04	0.886	330.0	285	0.863	29464
A3	3.01	0.887	327.9	284	0.867	29277
B1	2.00	0.807	396.1	349	0.881	35366
B2	2.00	0.743	363.7	360	0.991	32473
B3	1.71	0.657	275.5	344	1.247	24598
C1	1.55	0.584	333.5	464	1.391	29777
C2	1.84	0.707	478.3	495	1.035	42705
C3	1.60	0.684	401.6	444	1.105	35857

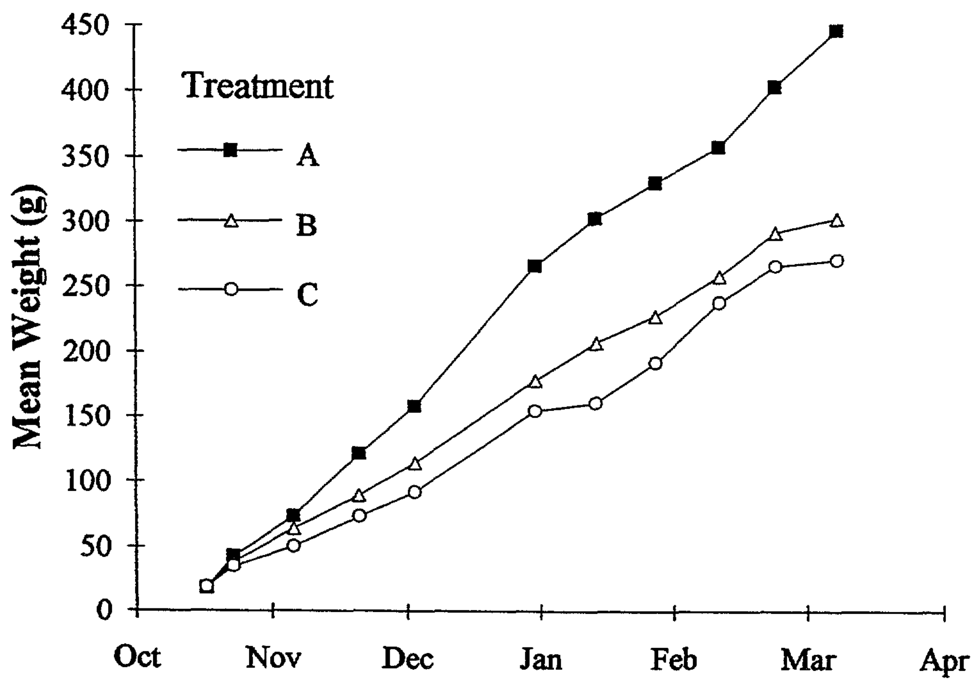

Figure 1. Changes in mean weight of tilapia during culture under 3 treatments; A = 3, B = 6, and C = 9 fish /m^2

Table 3. Multiple regression results for main effects (density) related to fish growth (g/d), survival (%), and yield (kg).

Variable	Coefficient	P
	Growth Rate ($r^2 = 0.811$, $p < 0.001$)	
Constant	3.42	0.001
Density	-0.210	0.001
	Survival ($r^2 = 0.739$, $p < 0.001$)	
Constant	0.984	0.001
Density	-0.038	0.002
	Yield ($r^2 = 0.281$, $p > 0.05$)	
Constant	267.39	0.001
Density	14.66	0.082

Results

Fish growth rate proceeded in a linear fashion throughout the experiment (Figure 1). Overall growth rate differed significantly among treatments (ANOVA, $p < 0.05$) with the low density treatment having higher growth than at intermediate density, which was higher in growth than the high density treatment (Tukey's test, $p < 0.05$, Table 2). Survival was also significantly different among treatments.

Feeding rate was initially high, then declined in all treatments (Figure 2). Overall feeding rate averaged 1.9%BW/d and did not differ significantly among treatments. Feed conversion rate averaged 0.89 and also did not differ significantly among treatments. Feed conversion rate varied over time, increasing to a peak after 45 days, then declining to zero in the intermediate and high density treatments.

Most physical and chemical variables showed no significant differences among treatments. Exceptions were chlorophyll-*a*, which was higher in the two high density treatments, and total volatile solids, which was higher in the high density treatments.

Growth rate was significantly correlated to density ($r^2 = 0.881$, $p < 0.001$, Table 3). Residuals of this regression were not significantly correlated to any chemical or physical variables. Survival was also significantly related to feed input ($r^2 = 0.739$, $p < 0.001$). Residuals of this regression were not significantly correlated to any physical or chemical variable. Finally, yield was not significantly related to density ($p > 0.05$).

Chlorophyll-*a* content differed among treatments (Figure 3), and was also strongly correlated to total volatile solids. However, there was no significant difference among treatments in primary production, which was measured less frequently than chlorophyll-*a*. Chlorophyll-*a* was strongly correlated to a number of physical and chemical variables which changed over the experiment. The strongest correlation was with total phosphorus ($r^2 = 0.701$, $p < 0.01$, Figure 4). Total phosphorus was also correlated to several physical and input variables; the most notable was feed input ($r^2 = 0.487$, $p < 0.001$, Figure 5). These results suggest that phosphorus was a limiting factor to primary production and was supplemented by high feeding rates.

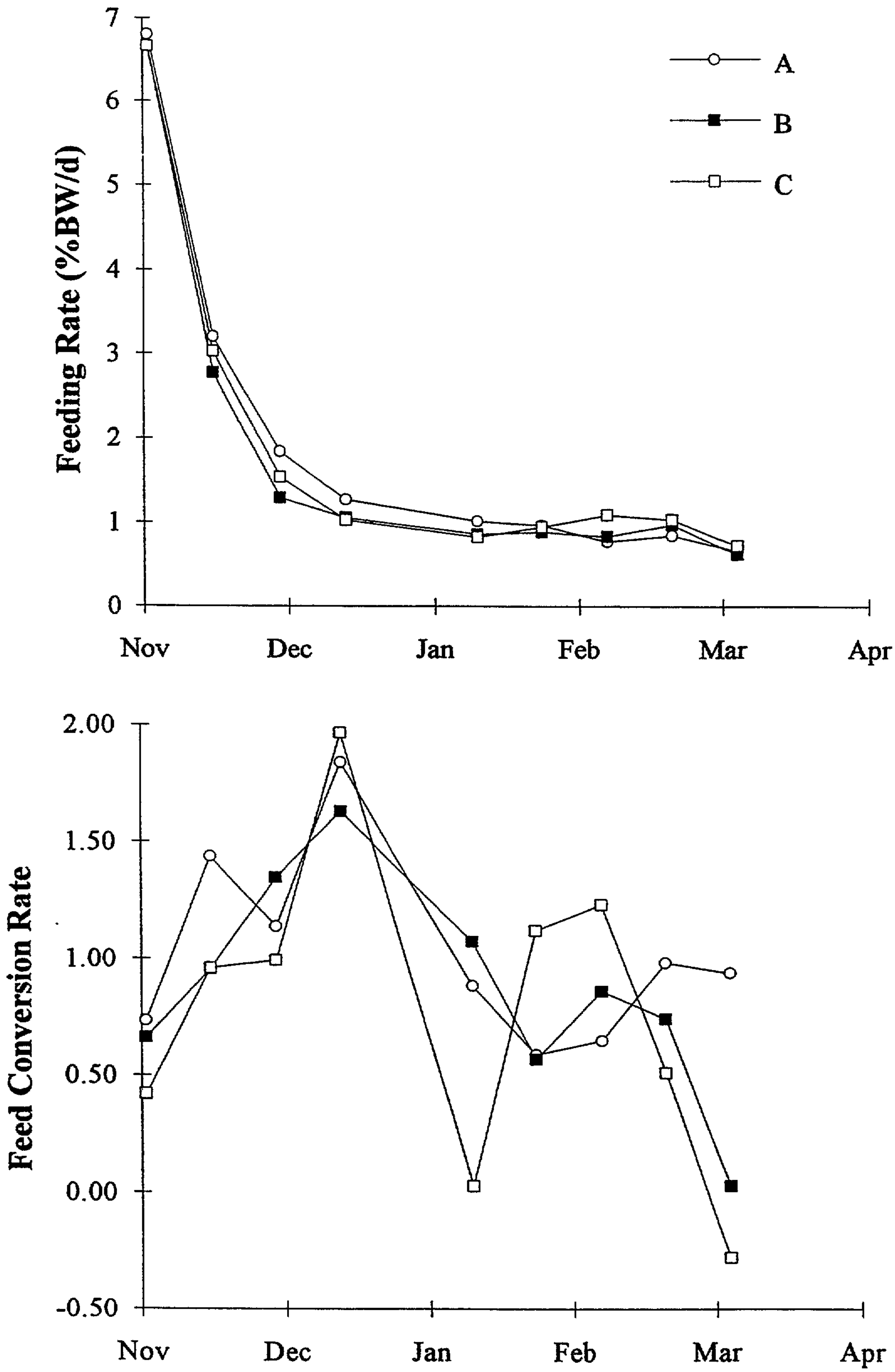

Figure 2. Changes in feeding rate and feed conversion rate during culture for ponds in each treatment.

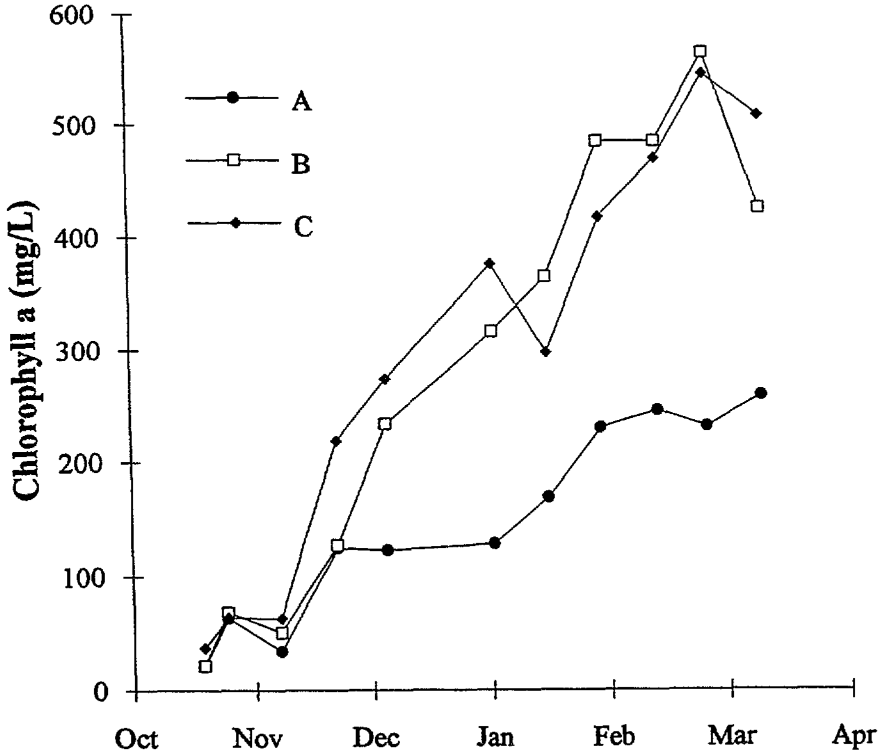

Figure 3. Changes in chlorophyll-*a* content of pond water during culture in each treatment.

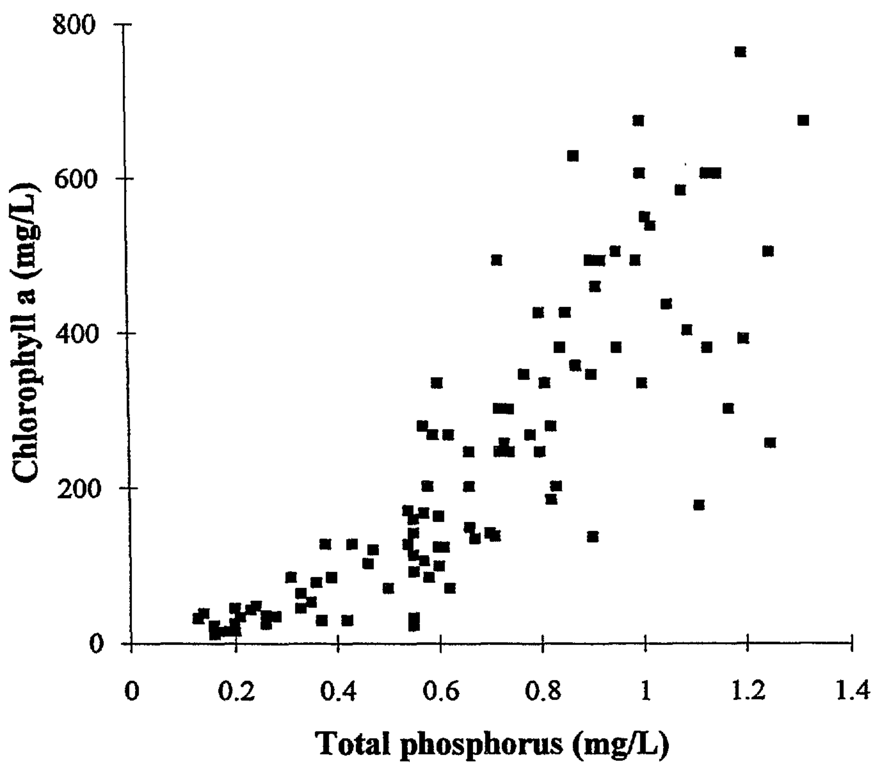

Figure 4. Relationship between chlorophyll-*a* content and total phosphorus in ponds of all treatments.

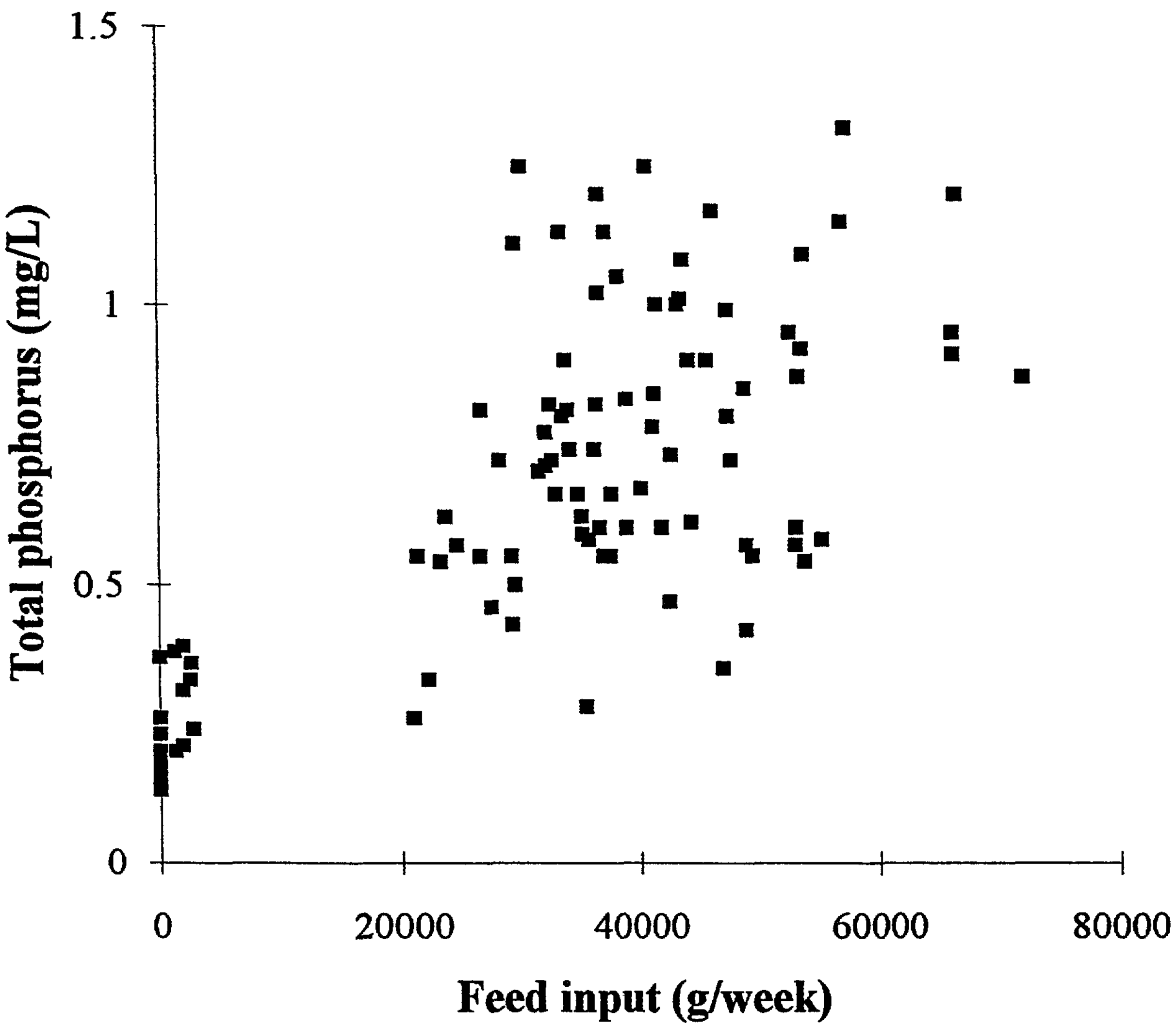

Figure 5. Relationship between total phosphorus and feed input in ponds of all treatments.

Discussion

Growth and survival of tilapia differed as expected among treatments, with best growth and survival at lowest density. Trends in growth rate among treatments were clearly differentiated by the first month sample. Growth was rapid in all ponds, and reached rates near the maximum measured for tilapia. However, reductions in growth which occurred at high density did not appear to be due to poor water quality, because water quality did not differ significantly among treatments. Thus, the reduced growth and survival in high density ponds appears to be a behavioral or physiological response to density itself, not to water quality. Supporting evidence was also obtained through statistical analysis, as the only variable that differed among treatments (chlorophyll-*a*) is a measure of natural food density, not poor water quality.

The density-dependent growth in this study was interesting because it occurred under ad-libitum feeding. Voluntary appetite suppression or behavioral interactions must have been involved in the reduced growth, or very subtle changes in water quality parameters may have influenced growth. Reduced growth due to declining water quality could be managed by aeration or water exchange, while behavioral or physiological reductions in growth defy management action.

Since growth declined at high density without concomitant declines in water quality, it is possible that feeding rates were too low in the high density ponds. However, there were no significant differences in feeding rates, which averaged 1.93, 1.78, and 1.87 %BW/d in the low, intermediate, and high density treatments, respectively. Because feeding rates were determined by actual food consumption, increased agonistic activity in high density ponds likely increased in energy expenditures and decreased growth rates. This was also somewhat expressed in feed conversion data, as the most efficient conversion occurred at lowest density, although differences among densities were not significant.

The application of this study to tilapia management is not entirely clear. Most rapid growth and highest survival occurred at 3 fish/m^2. Since increased densities required more feed to achieve the same yield, culture at these densities was inefficient. However, feed conversion values were not significantly different among treatments, contradicting the former statement. The optimal feeding system at present appears to be with tilapia at 3/m^2. The combined application of feed and fertilizer remains an important tool, as even at 9 fish/m^2 with intensive feeding, water quality remained high. However, the enhanced chlorophyll-*a* levels which occurred in heavily fed ponds appeared to result from surplus phosphorus in the water, and may indicate that further fine-tuning of fertilizer balance may be necessary at high feeding rates.

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Diel Cycles of Temperature and Dissolved Oxygen Stratification in Deep Rain-fed Ponds

Work Plan 7, Thailand Study 7

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Introduction

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 constructed a set of experimental ponds on the Huay Luang Fisheries Station of the Royal Thai Department of Fisheries.

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. Manage-ment 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 optima, 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. The objectives of this work are: 1) to describe and quantify diel cycles of temperature and DO stratification in deep rain-fed ponds; 2) to compare these patterns with those of shallower ponds typical of CRSP experiments; and 3) to apply this information in the context of practical pond management.

Materials and Methods

Ponds of 800 m^2 area and 2.5 m depth at the Huay Luang station were monitored during fish growth experiments designed by the University of Michigan component. Diel cycles of temperature and DO were recorded with an automated monitoring system similar to that used at AIT, and described by Szyper and Lin (1990), Green and Teichert-Coddington (1991), and Szyper and Ebeling (1993). Temperature was monitored by thermocouples deployed on a plastic pipe suspended from a float; DO was assessed in water pumped from the pond through a receiving

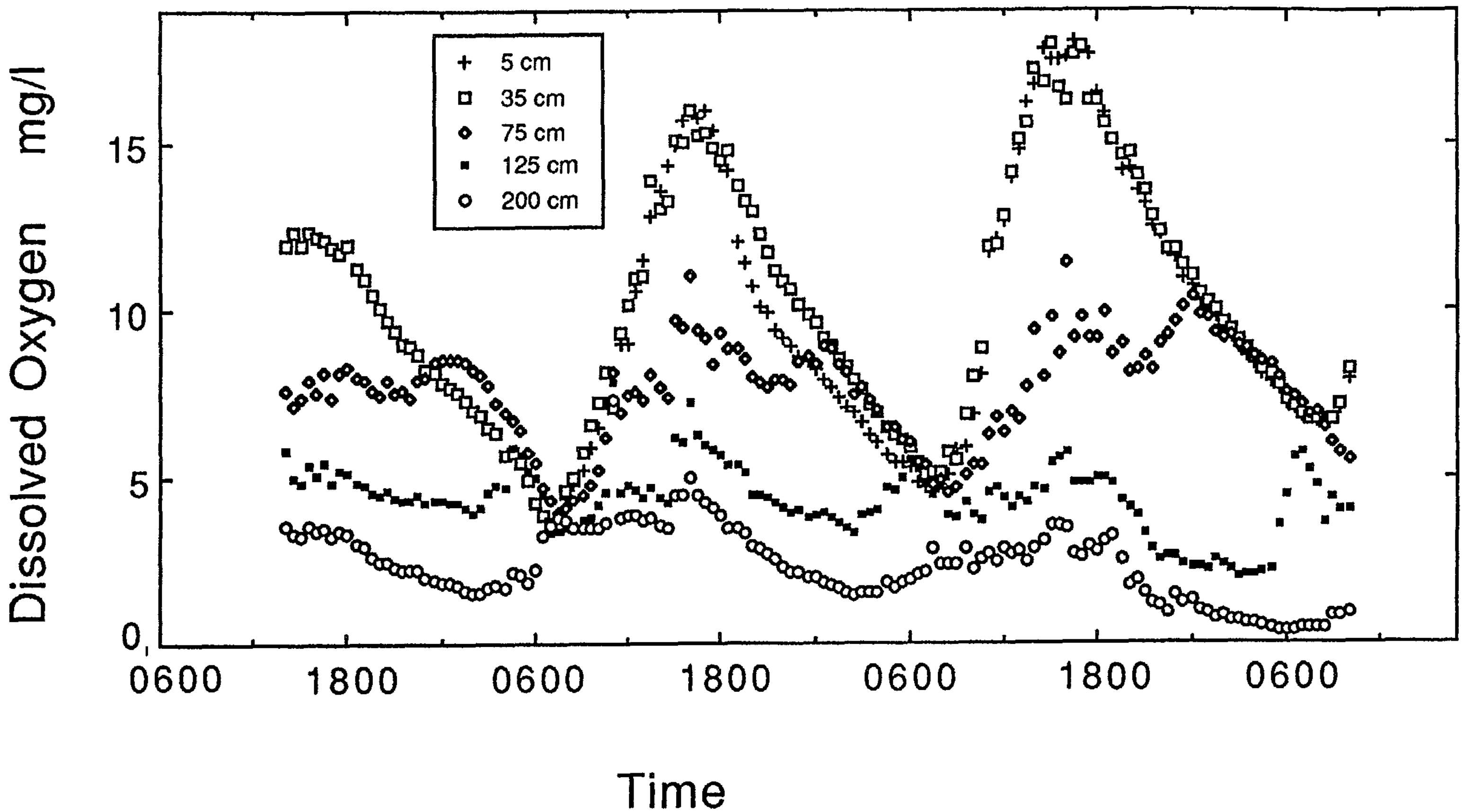

Figure 1. Several days' diel cycles of dissolved oxygen concentration at five depths in an earthen pond of 2.5 m depth at the Huay Luang Fisheries Station in Northeast Thailand during the dry season.

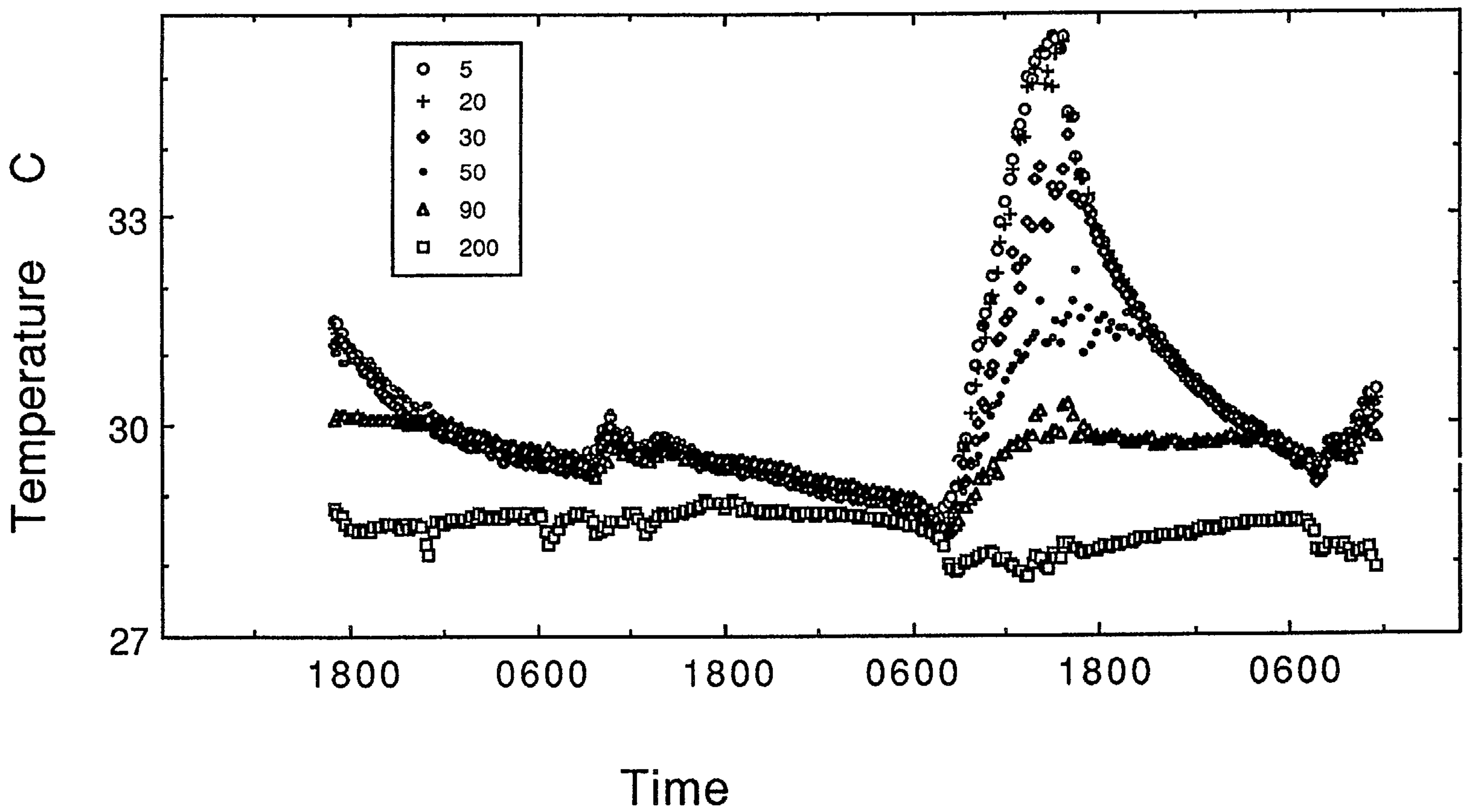

Figure 2. Several days' diel cycles of temperature at six depths in an earthen pond of 2.5 m depth at the Huay Luang Fisheries Station in Northeast Thailand during the rainy season. The center of the plot shows the cycle on a cloudy day; sun shone the next day.

chamber of plastic pipe on the pond bank at intervals of 30 minutes. The system was modified for convenient pump sampling of five depths as described by Jiang (1994).

Results and Discussion

Diel cycles of DO in the deep ponds on two successive dates during November (dry season) are shown in Figure 1. The oxygen curves overlap for the 5 cm and 35 cm depths, indicating a mixed layer of at least the latter depth. Wind speeds are not recorded at this station, but were qualitatively greater in general than at the AIT pond facility, where the mixed layers are generally of 10-20 cm depth on sunny days. At 75 cm, there is substantial isolation of this intermediate depth from the surface layer; the scatter of the points indicates some mixing on the time scale of hours, however. Convective mixing after sunset appears to reach to at least 125 cm, but not to 200 cm. At AIT, Szyper and Lin (1990) found the 1.2 m depth to be continuously isolated from the upper layers throughout the diel period when days were sunny. The figure also shows gradually declining values of DO at the two deepest depths. The ponds had been completely mixed the previous evening due to the work of installation of the apparatus. The average slope of the 200 cm plot (0.1 mg $l^{-1}h^{-1}$) could be taken as an estimate of the in situ respiration rate if the isolation of this layer were more nearly perfect. However, the slight increase in DO during the daylight hours of the second date, as well as the approach of the upper depths' curves to the lower one at night, indicate some transport of oxygen to this depth, and so the rate of decrease here would underestimate community respiration.

Temperature cycles recorded during the rainy season (August, Figure 2) again show the isolation of the 200 cm depth, in this case through the afternoon and night of one partly cloudy day, the following rainy day without clear sunshine, and the succeeding sunny day. Thus, despite the more vigorous mixing by wind which is characteristic of this site, there will still be, in deep ponds, an isolated deep layer of considerable thickness, at 1.5 to 3 meters depth, in addition to the cyclical DO depletion at intermediate depths. This condition was not alleviated by the occurrence of dark rainy days, during which the pond remained isothermal down to 90 cm, but the 200 cm depth was still isolated.

If a pond of 3 m depth has its deepest 1.5 m isolated and depleted of oxygen, then a complete mixing, as could occur in the evening of an unusually windy day following little solar radiation, could rapidly halve the DO concentration of the upper layer for the final concentration. While this might not in itself endanger fish which are tolerant of low DO, it does increase the chances of whole pond depletion by the following dawn. In addition, a constantly depleted deep layer could slow the growth of characteristically bottom-foraging or bottom-resting fishes.

The assessment techniques used here are now available to the entire aquaculture work group at AIT, including outreach specialists. These techniques will be useful for measuring the effects on bottom isolation of various low-cost mixing strategies, including those described by Szyper (1995).

Acknowledgments

We appreciate the collaboration of the University of Michigan, the Royal Thai Dept. of Fisheries, and the Asian Institute of Technology. Thanks are due to the AIT Aquaculture Laboratory for technical support, and to Messrs. Manoj Yomjinda and Arun Patel for field and laboratory work.

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Carbon Dioxide Exchange between Pond Water and the Atmosphere

Work Plan 7, Thailand Study 8

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Introduction

Rates of exchange of dissolved oxygen 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 1977, 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 reported estimates of carbon dioxide exchange rates have been made under conditions of less severe density stratification and lower rates of primary production than is typical of CRSP experiments or of fed production ponds. There are indications that carbon dioxide exchange rates are too large to neglect, though they are somewhat smaller compared with photosynthesis and respiration than those observed for oxygen (Szyper and Ebeling 1993). Good estimates of these rates in typical CRSP ponds would 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.

The objectives of this work were to quantify the rates of exchange of carbon dioxide between pond water and the atmosphere in fertile earthen ponds, and to elucidate major factors which determine these rates.

Materials and Methods

This study could not be completed at the site which was originally projected (AIT) because the monitoring system could not be made to perform all functions required during the project period, and the wind speed records from the AIT weather station could not be rationalized to the required time intervals. The objectives were attained, however, by analysis of data from the University of Hawaii pond research facility in the U.S. These are the PI's original data and have not been presented elsewhere. Summary totals for some of the quantities were presented and discussed in Szyper and Ebeling (1993), where the monitoring system is also described in detail. 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 (dissolved molecular, or "free") carbon dioxide, carbonic acid, bicarbonate ion, and carbonate ion, is calculated from pH, temperature, and total alkalinity (the latter analyzed manually). Primary production and

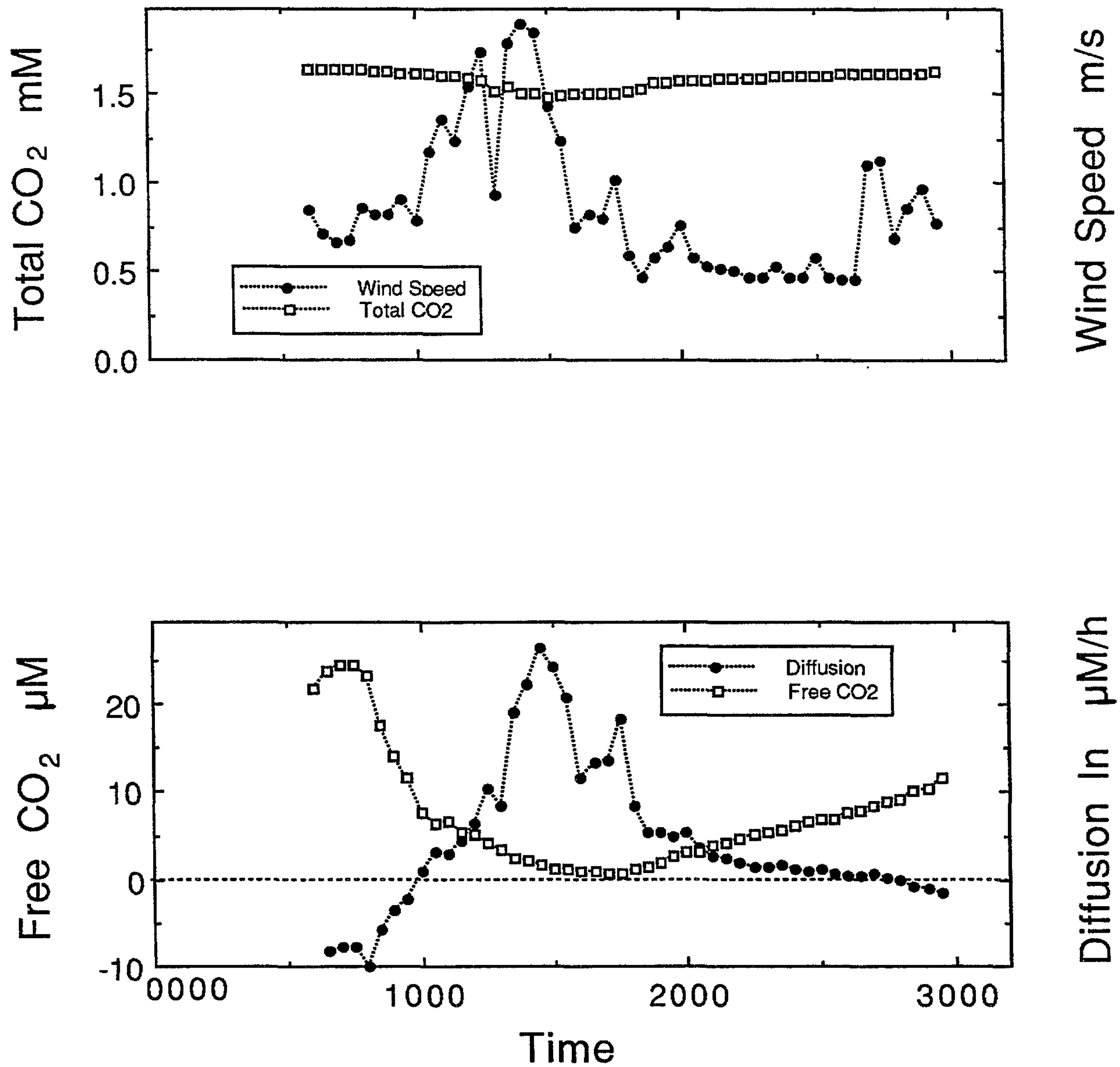

Figure 1. Typical sunny day, heavy-bloom condition diel cycles of Total CO_2, Free CO_2, and rates of CO_2 diffusion into or out of the atmosphere, observed (along with wind speed) in a fertile brackish water earthen culture pond.

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 and Discussion

Figure 1 shows typical diel cycles in a brackish water earthen pond of 2000 m^2 area, 0.7 m depth, salinity of about 15 ppt, with a bloom of small coccoid cyanobacteria at chlorophyll a concentration of about 400 mg per liter. As expected, particularly in a brackish pond, total CO_2 concentrations varied little during the day, but did show a perceptible dip during midday reflecting photosynthetic uptake. Wind speeds at 0.5 m above water surface ranged from about 0.6 to 1.8 m/s, with the windiest periods concentrated into daylight hours, in this case beginning at 1000 hours. The "Free CO_2" fraction of total CO_2, amounting to about 1% of the total, (note the different vertical scales) varied in parallel with total CO_2, but accounted for only about 25% of the diel variation in total CO_2. Although only free CO_2, of all the total CO_2 components, can diffuse as a gas, the system responds to addition or removal of free CO_2 by conversion of the other forms to restore equilibrium.

Diffusion of CO_2 into or out of the water (here indexed with the rate of diffusion *into* the water taken as positive) is expected to depend primarily on wind speed and concentration of free CO_2, but will be affected by temperature and other factors. Before 1000 hours, free CO_2 was diffusing out of the water, with the diffusion rate accounting for much of the decline in concentration, but photosynthetic uptake may have contributed to the decline. After 1000 hours and until late afternoon, CO_2 diffused *into* the water due to low concentrations and increased wind speed, but the concentration continued to decline to near zero, now certainly reflecting photosynthetic uptake. The actual CO_2 species taken in by plant cells varies under different conditions and is beyond the scope of this report. "Photosynthetic uptake" here refers to any form of DIC, and it is presumed that rapid equilibrium adjustments in the distribution of CO_2 species occurs. On this date, daytime net primary production (carbon fixation) was 0.89 g C m^2; diffusion into the water column during daylight hours was 0.24 g C m^2, consistent with Szyper and Ebeling's (1993) report that diffusion typically amounted to about 30% of production during the two week study period which included this date. It should be noted, however, that diffusion was relatively much more important on days of low production.

A multiple regression analysis was made without consideration of the temperature cycle, presuming that the primary effect of increased daytime water temperatures would be to cause diffusion *out of* the water, whereas in fact diffusion was predominantly *in* during daytime hours. The analysis showed that the concentration of free CO_2 and wind speed together accounted for 81 % of the variation in the diffusion rates during this diel cycle; each of these factors was significant at a << 0.01. The net rates of change in CO_2 concentration during each 30 minute sampling interval (d DIC/dt) represent net photosynthetic uptake during daylight hours (gross uptake plus release by community respiration), and community respiration at night. This factor contributed no significant effect to diffusion rates and did not add to the percentage of the diel variation in diffusion rates which was explained by the other two factors. Model prediction of diffusion rates thus requires only observed concentrations and wind speed, though the above discussion shows that photosynthetic demand can be the primary determinant of concentrations under some conditions.

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Growth Comparison of Three Strains of Nile Tilapia in Fertilized Ponds

Work Plan 7, Thailand Study 9

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Introduction

Three preliminary experiments using sex-reversed Freshwater Aquaculture Center (FAC) *Oreochromis niloticus* and genetically male tilapia (GMT) produced by breeding YY- male with female Egypt-Swansea *O. niloticus* were conducted from 1991 to 1994 (Hopkins et al., 1994). In those experiments, the yields attained with FAC *O. niloticus* were considerably lower than the yields of GMT Egypt-Swansea fish and were lower than yields of Thai *O. niloticus* at the CRSP project at the Asian Institute of Technology in Bangkok. Also, the net yield of FAC *O. niloticus* at fertilizer rates of 14 kg N/ha/wk and 28 kg/ha/wk were not significantly different even though linear relationships between N inputs and yield were observed in Thailand (Szyper et al., 1991). Two major questions were raised by these observations. First, is the growth rate of FAC *O. niloticus* less than the growth rate of Thai *O. niloticus*? Second, do different strains of tilapia have differential abilities to utilize the increased primary productivity induced by the higher fertilizer rates? The objective of this study was to answer those questions.

Materials and Methods

Fingerlings of three strains of *O. niloticus* were grown in twelve 500 m^2 ponds at the Freshwater Aquaculture Center (FAC) located on the campus of Central Luzon State University, Munoz, Nueva Ecija, Philippines. The FAC strain is descended from *O. niloticus* imported to the Philippines in the 1970s and kept at FAC. These fish are believed to be introgressed with wild *O. mossambicus*. (Pullin, 1988). The Thai strain was imported from Thailand in the 1980s for breeding by the Bureau of Fisheries and Aquatic Resources and ICLARM (Eknath et al., 1993). The Egypt-Swansea strain originated with fish collected from Lake Manzala in 1979 that were used to create a laboratory stain at the University College of Swansea during the 1980s. Several adults and several hundred fry from this laboratory stock were subsequently transferred to FAC in 1979 as part of a project to develop YY-male tilapia. These fish, like the Thai strain used in this study, are carefully maintained and monitored.

The FAC and Thai fingerlings were sex-reversed with methyl-testosterone before stocking. The Egypt-Swansea fish were not sex-reversed because they had been produced by crossing YY-males with normal females, thereby yielding only genetically-male tilapia (GMT). Fingerlings were stocked at 2 fish per m^2 and at an initial size of 3-4 g each. The culture period was 137 days.

Two inorganic fertilizers, urea and ammonium phosphate, were applied once a week on Wednesdays. The fertilizers were mixed with water and then broadcast into the ponds. The nitrogen fertilizer rate was 4 kg N/ha/d in three treatments (3 ponds per strain = 9 ponds) but was reduced to 2 kg N/ha/d in a fourth treatment with Egypt-Swansea fish (3 ponds). The N:P ratio was 5:1 in all treatments.

Water supply was from a well. Water depths were maintained at approximately 1 m. Makeup water was added weekly to replace seepage and evaporation. Initial alkalinity were approximately 200 mg $CaCO_3$/liter. The average water temperature was 30°C.

Complete counts of all the fish were made at stocking and harvest. The bulk weight of all fish was taken at harvest. Sample weights were determined monthly throughout the culture period.

Results and Discussion

The extrapolated fish yields (mean + s.e.) in ponds receiving 4 kg N/ha/d were:

FAC strain: 2389 + 251 kg/ha/yr
Egypt-Swansea strain: 5265 + 514 kg/ha/yr
Thai strain: 4991 + 418 kg/ha/yr

There was no significant difference between the Egypt-Swansea and Thai strains while the FAC strain showed a significantly lower yield. When the nitrogen fertilization level was reduced to 2 kg N/ha/d, the yield of Egypt-Swansea strain fish was 4675 + 666 kg/ha/yr. This yield was not significantly different from the yield attained with 4 kg N/ha/d.

This experiment verified earlier observations that the FAC strain had significantly lower growth performance than the Egypt-Swansea strain. Also, the poor performance of the FAC strain versus the Thai strain offers an explanation for why earlier studies at FAC had lower yields than those attained in Thailand with similar fertilizer rates. Another concern with the FAC fish is that average yield with this strain appears to be rapidly decreasing. In early 1992, extrapolated yields averaged 4691 kg/ha/yr. In late 1992, they were approximately 4069 kg/ha/yr. In 1993, the yield was 3941 kg/ha/yr. But in the current study, the yield of FAC strain was only 2389 kg/ha/yr. The obvious conclusion to be made from these results is that the FAC strain needs to be replaced by higher yielding strains.

Of equal importance to the economic sustainability of these systems is the observation that the yields attained with 2 kg N/ha/d were equivalent to those attained with 4 kg N/ha/d. This result with Egypt-Swansea strain had been observed previously with FAC strain. The result appears to be quite different from the results of experiments in Thailand in which 4 to 5 kg N/ha/d provided significantly higher yields (Szyper et al., 1991). The reason for these disparate results needs to be determined.

Acknowledgments

We greatly appreciate the collaboration of Dr. Graham Mair, University College of Swansea, who provided the Egypt-Swansea strain from stocks maintained at FAC.

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Effects of Pond Size

Work Plan 5, Thailand Study 9

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Introduction

Having studied the relationship of pond depth to fish production and other parameters of ecosystem response to fertilization (Szyper et al., 1991; Szyper and Hopkins, 1993), the Thailand component next investigated the effects of pond surface area. Extrapolation, particularly scaling upward the results of trials in small impoundments to production scale ponds is a classical and unsolved problem in aquaculture. There are numerous cases of impractical, high areal production rates observed in research scale water bodies (Colman and Edwards, 1987). Our interest here was not in "solving" the larger problem, but rather to examine earthen ponds of available different sizes for potential effects on CRSP experimental results at the Asian Institute of Technology (AIT). The results should have more general applicability because the relatively small ponds used (areas of 200 to 1400 m^2) are similar to those used by farmers in Thailand, the Philippines, and elsewhere, though farmers' ponds range to much greater areas.

Materials and Methods

A five-month experiment was conducted in 11 earthen ponds of four different surfaces areas (approximately 200,380,610, and 1390 m^2) with triplicate ponds of each size, at AIT. Ponds were stocked with sex-reversed fingerlings of Nile tilapia, *Oreochromis niloticus*, at 2 fish per m^2, maintained weekly to depth of 1.0 m, and fertilized weekly with chicken manure at 250 kg dry matter/ha/wk supplement with urea and TSP to attain rates of 35 kg N/ha/wk and 7 kg P/ha/wk. Water sampling and analysis were performed according to standard protocols, with detailed water sampling/analyses conducted every month.

Results and Discussion

Extrapolated yields ranged from 1,921 to 8,631 kg/ha/yr (Table 1). Single-factor ANOVA showed no significant relationship between yield and pond size. A plot of yield as a function of pond size (Figure 1), although positive, was also not significant.

Table 1. Extrapolated Fish Yields (kg/ha/tr)

	Pond Size (m^2)			
	200	380	610	1390
Replicate 1	4,918	6,905	7,709	8,631
Replicate 2	3,520	1,921	4,896	4,670
Replicate 3	3,923	5,277	6,714	
Mean	4,120	4,701	6,440	6,651
Standard Error	416	1,467	824	1,981

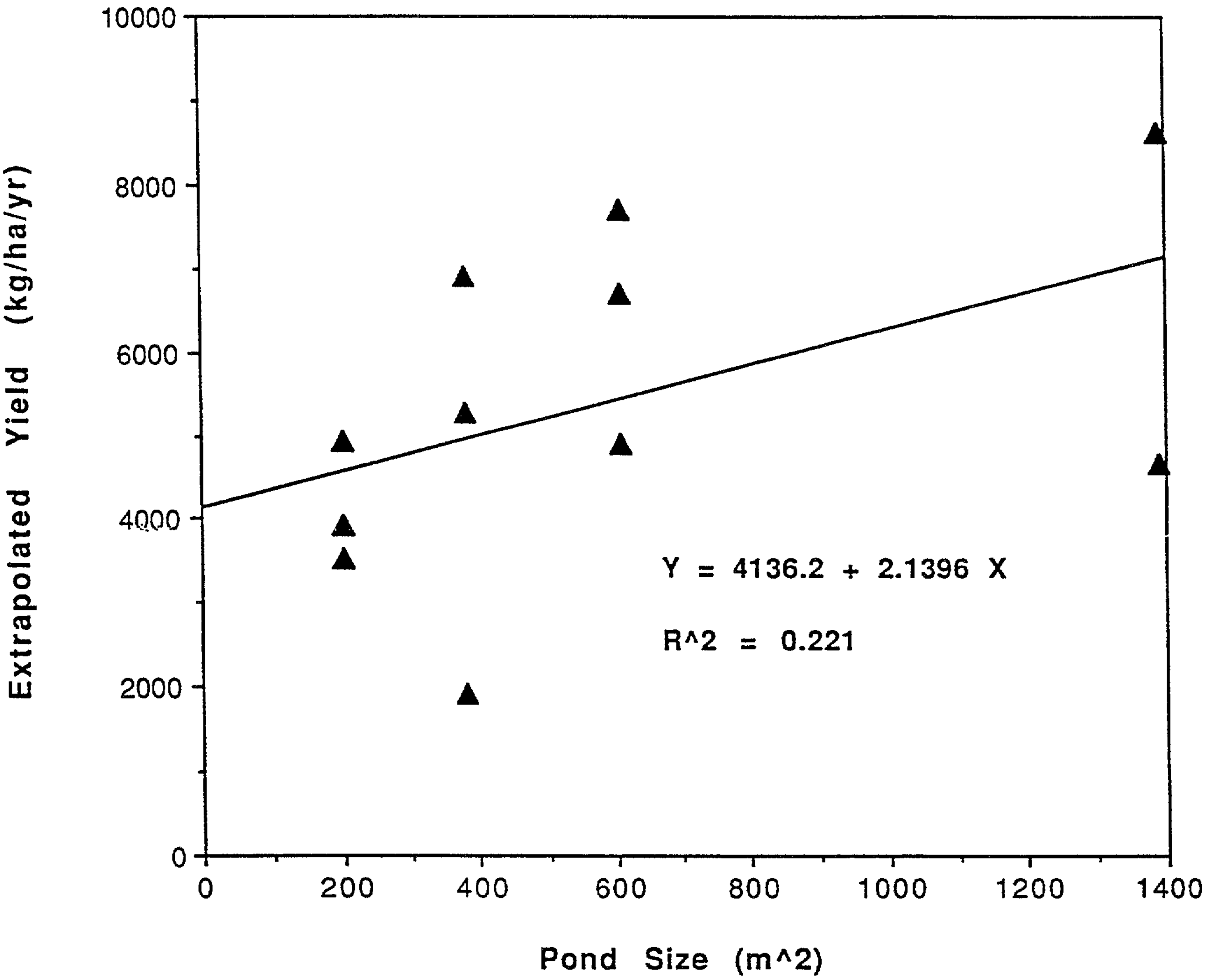

Figure 1. Relationship between pond size and yield.

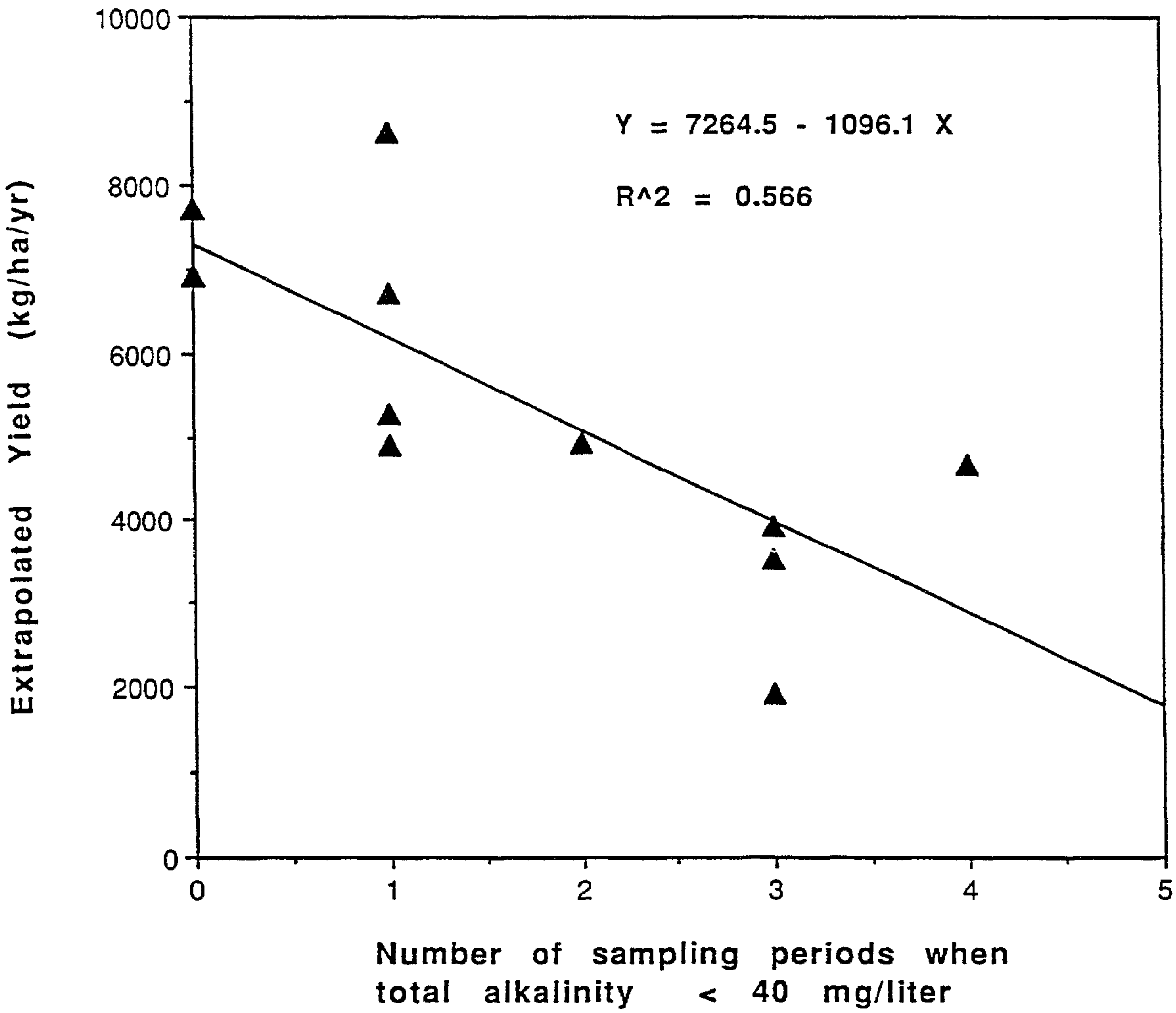

Figure 2. Relationship between alkalinity and yield.

However, further analysis indicated low alkalinity (Figure 2). Computing a multiple regression relating pond size and low total alkalinity to fish yield produced the following equation:

$$Y = 5988 + 2.34\ S - 1137\ C$$
$$R^2 = 0.83$$

where Y= extrapolated yield (kg/ha/yr), S = pond size (m^2), C = the number of monthly sampling periods in which the total alkalinity was below 40 mg/l, and R = coefficient of determination. Both S and C were highly significant (a = 0.01).

These results disprove the null hypothesis of no significant effect of surface area, within the range examined, on fish production under this fertilization protocol. This means that areal fish yields may be expected to increase as ponds become large. However, extrapolation to pond sizes beyond the largest used here is unwarranted.

It is not clear, as noted, why yield results from very small containers characteristically cannot be extrapolated. Larger ponds present greater linear dimensions to the wind per unit surface area which should cause them to be more easily or completely stirred by the wind. This in turn might enhance production which begins with microbial food chains (Costa-Pierce and Pullin, 1989). A short-term study conducted simultaneously in these ponds suggested that larger ponds were also mixed more easily (energy-efficiently) by artificial devices when they were strongly density stratified (Szyper, 1995), but this factor was not tested for its effect on the longer term production.

Acknowledgments

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United States: Data Analysis and Synthesis

The Data Analysis and Synthesis Team (DAST) at the University of California, Davis (UCD) continued refining several pond models reported on in previous annual reports and publications. For example, a model designed to simulate temperature and dissolved oxygen concentrations in stratified ponds was modified by using stochastically generated weather parameters as inputs. The model can be executed for an 85-day simulation, and work is underway to increase the number of time-steps the model can process. Results indicate that temperature and dissolved oxygen predictions from the model match well with measured values; however, fish growth and chlorophyll-*a* concentrations are consistently overestimated.

Another study at UCD involves developing a preliminary model to investigate the effects of integrated aquaculture and agriculture on nutrient cycling and whole system productivity. The model will concurrently evaluate the impacts of various management actions for enhancement of pond sediment quality. The model consists of three modules: fishpond, crop, and terrestrial soil nitrogen. Inputs of nitrogen into the pond include feed and/or fertilizer and water. Outputs from the pond include uptake by fish, effluent water, and removal of pond sediments. The three modules are linked through the use of sediment from ponds as crop fertilizer and/or the use of wastes from crops as feed/fertilizer to aquaculture ponds. Preliminary results demonstrate that feed quality and digestibility of feed need to be considered to improve overall estimation of organic matter and nitrogen production in the fish pond, and to improve estimation of fish growth.

The Data Analysis and Synthesis Team (DAST) at Oregon State University further refined the decision support system *POND*. In a study to demonstrate that *POND* can be readily adapted to different species and/or culture conditions, OSU DAST researchers parameterized the fish bioenergetics for *Ictalurus punctatus* (channel catfish), *Colossoma macropomum* (tambaquí), and *Piaractus mitrei* (pacu). Simultaneous validation of the water temperature model for CRSP and non-CRSP locations was also completed in this study. A second study was implemented to examine the use of genetic algorithms for estimating suitable bioenergetic parameters for different pond species. The reports, *Decison Support for Pond Aquaculture: Simulation Models and Applications* and *Decision Support for Pond Aquaculture: Parameter Estimation for Simulation Models*, respectively, are contained in the Global Studies and Activities section of this pulication.

Aquaculture Pond Modeling for the Analysis of Integrated Aquaculture/Agriculture Systems

Interim Work Plan, DAST Study 2

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Introduction

Increased adoption of new activities such as aquaculture into existing agroecosystems calls for the application of simulation models to analyze and forecast consequences of new agroecosystem designs (Elliot and Cole, 1989; Edwards et al., 1988). The main objective of integrated systems is to enhance nutrient cycling and energy flow in the system to obtain maximum benefits in the production of food and fiber (Chan, 1993). Integration of aquaculture and agriculture through the use of pond sediment organic matter as a crop fertilizer, and of pond water for irrigation, establishes linkages between aquaculture ponds and crops.

The large body of literature on aquaculture pond nutrient budgets shows that pond sediments are a major sink of nutrients accounting for 65-72% of nitrogen supplied to ponds (Acosta-Nassar et al., 1994; Briggs and Funge-Smith, 1994; Olah et al., 1994; Schwartz and Boyd 1994). Management actions like feeding rates, feed types, organic matter input and fish species reared may affect pond processes such as organic matter settling, resuspension, nitrification, ammonification, and hence quality of sediments. Therefore, sediment-water nitrogen processes, nutrient recycling, resuspension and nitrogen retention in aquaculture ponds are likely to be important in integrated system models.

Energy and nutrient cycling studies have attributed the observed sustainability of integrated systems to high intrasystem nutrient and material cycling (Ruddle and Zhong 1984; Soemarwoto, 1974). However, integrated systems have not been adequately studied because of their complexity (Edwards et al., 1988). In addition, conventional tools for agroecosystem analysis like energy budgets, do not capture the dynamic properties of the systems (Lightfoot et al 1993; Conway 1987). Simulation models are useful tools in the analysis of complex systems and biogeochemical cycling of nutrients (Anderson, 1992; Thornley and Verbene, 1989). Although system modeling techniques are important for future research in agroecosystems, they have yet to be applied to integrated systems having an aquaculture component (Edwards et al., 1988).

The objectives of the work described in this report is to develop a computer model that can be used to analyze and predict nitrogen and organic matter outputs from an aquaculture pond by modifying current pond ecosystem models to explicitly include organic matter and nitrogen processes. The model developed will be linked with an agriculture/crop model, and the resulting integrated model will serve to simulate the flow of organic matter and nitrogen through combined aquaculture and conventional agriculture practices.

Model Structure

The model consists of three primary modules: Fish Pond, Crop, and Terrestrial Soil Nitrogen (Table 1). In turn, each primary module includes several submodels containing state variables describing the system.

The fish pond module is based on worked carried out by the OSU and UC Davis DAST (e.g. Bolte et al., 1994; Giovannini, 1994; Giovannini and Piedrahita, 1994; Culberson, 1993; Piedrahita, 1990). The crop module is primarily based on SUCROS, a general crop growth model (van Kuelen et al., 1982). Soil nitrogen transformations and water balance equations will be added to the crop module to simulate soil organic matter dynamics, nitrogen availability and uptake by crop. Figure 1 shows in a relational diagram how different submodels interact to simulate fluxes and pools of materials and nitrogen. Details of the area in which work over the last year has focused are presented below.

Table 1. Primary models, submodels, and state variables for an integrated aquaculture-agriculture nitrogen dynamics model

Model	Submodel	State Variables
Fish pond	Fish growth	fish biomass, phytoplankton biomass
	Phytoplankton	phytoplankton biomass
	Water quality	water column organic matter, fish biomass
	Feed quality	feed (artificial feed and algae) N concentration
	Feed uptake	
	Sediment	sediment nitrogen and organic matter concentration
Crop	Crop growth	soil nitrogen, soil water content
	N uptake	
Terrestrial Soil	Organic matter	detrital biomass
	soil nitrogen	soil organic matter, crop biomass, soil water content

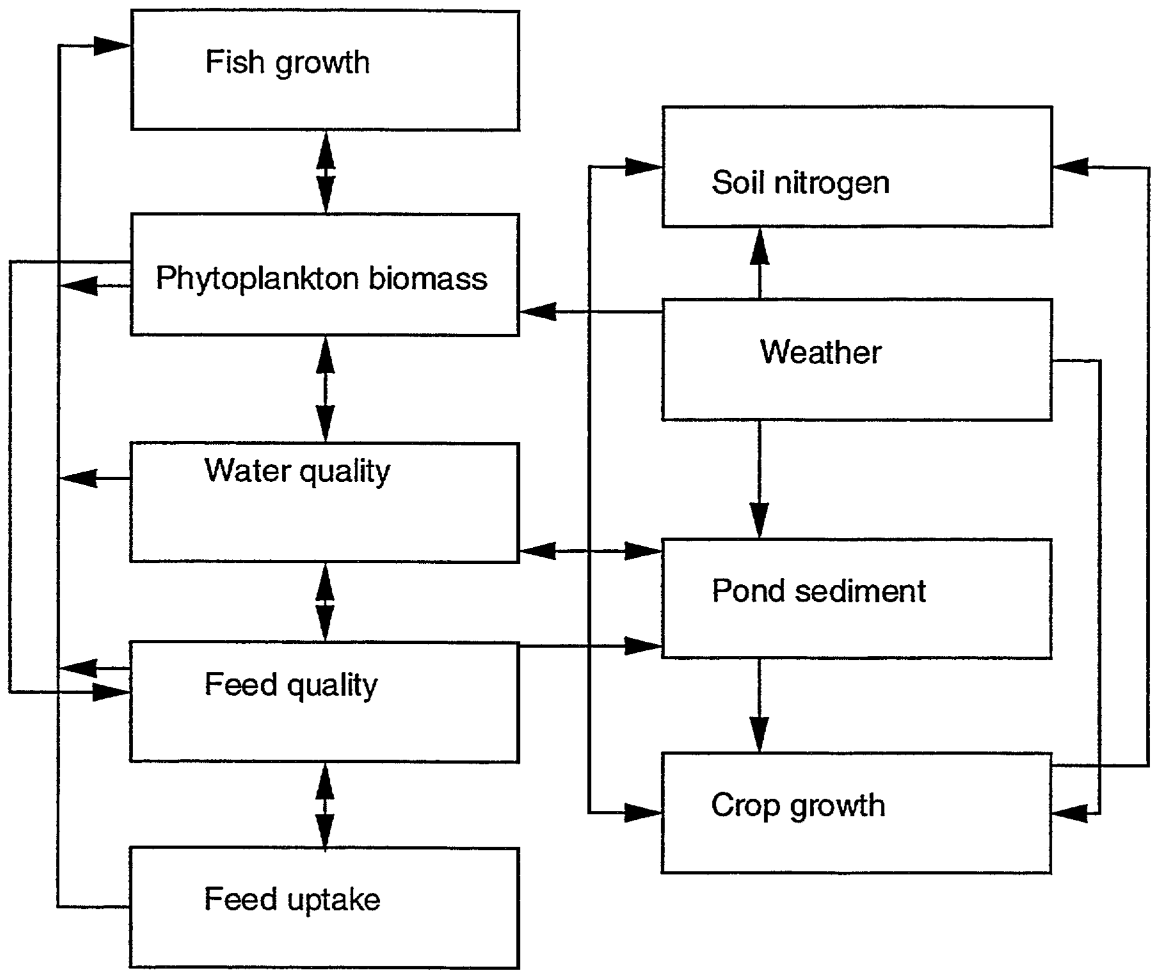

Figure 1. A relational diagram showing connections and feedback between different modules in the nitrogen dynamics model for integrated aquaculture-agriculture systems.

Fish Growth

The fish growth module is adapted from a model developed by the OSU DAST (Bolte et al., 1994). The model describes the growth rate of an individual fish using a differential equation (Ursin, 1967). The OSU DAST model has been modified to include effects of feed uptake from artificial feed and/or phytoplankton on feed quality and feed digestibility. The modified differential equation for fish growth in the new model is:

$$\frac{dW}{dt} = (1-a)q\sum_{i=1}^{k} b_i \frac{dR_i}{dt} - k_{min}e^{[s(T-T_{min})]}W^n \quad (1)$$

$$\frac{dR_i}{dt} = hf\tau\delta W^m \quad (2)$$

where
W = weight of fish (g)
t = time (d)
a = fraction of food assimilated that is used for catabolism (unitless)
q = coefficient describing the effect of feed quality on fish growth (unitless)
b_i= efficiency of assimilation for ith feed resource
R^i = intake rate of feed i (g/d)
h = coefficient of food consumption (g^{m-1} /d)
f = relative feeding level (unitless)
τ= temperature parameter (0 to 1);
δ = function describing the effects of DO or unionized ammonia on food intake (0 to 1)
m = exponent of body mass for anabolism (g^{1-m}/d)
k = coefficient of catabolism
s^{min}= constant (°C^{-1})
T = water temperature (°C)
n = exponent of body mass for catabolism (g^{1-n}/d) at the minimum temperature for the species for the species T_{min} and coworkers (1994), except for the intake rate and coefficient of feed quality.

The coefficient of feed quality (q) is a new parameter introduced in the model presented here. This parameter has been added so that the effect of variable feed quality on fish growth can be simulated. This is necessary because changes in artificial feed types and feeding rates will lead to changes in the diet composition of fish, and will ultimately affect fish growth. The feed intake rate term ($\frac{dR_i}{dt}$) has been modified also, and now incorporates separate food assimilation coefficients (b) for each feed resource instead of an average value for all feed resources in the pond. In addition, feed intake rates from a particular feed resource are calculated based on the assumption that fish will prefer artificial feed regardless of the concentration of other feed resources. This approach is different from that adopted in POND, where feed intake rate of a particular feed resource is calculated using Michalis-Menten kinetic models. In this approach, feed uptake is dependent on the maximum possible uptake, a preference factor (half saturation constant) and the feed concentration. Experimental evidence (e.g. Brummett, 1994; Schroeder, 1978) suggest that tilapias prefer artificial feed to natural feed under culture conditions. Therefore, an assumption is made for the model that fish will take artificial feed independent of the concentration of natural feed resources. Whenever, fish cannot meet their daily feed intake requirements from artificial feeds, they will supplement their feeding by consuming natural foods. A ratio of actual to optimal feed rates (artificial feed factor, 0 to 1) will be used to model feed uptake from multiple feed resources. The artificial feed factor will be calculated as follows: if actual input rates of artificial feed are greater than or equal to optimal feeding rates for that particular size class of fish, the artificial feed factor is one. If the artificial feed factor is less than one then fish must feed on plankton in addition to artificial feed to satisfy their daily feed intake on the basis of bioenergetic considerations. The plankton feed factor is then defined as one minus the artificial feed factor. The optimal feed rate is based on standard feed rates from the literature, whereas actual feed rate is a decision variable which depends on management considerations. The effect of feed quality on growth will be incorporated in the model using Sterner and Hessen's approach (1994):

$$q = \frac{N:C_F}{K_c(N:C_Z)} \quad \text{if} \quad Q^*_{C-E} > N:C_F \quad (3)$$

or

$$q = 1 \quad \text{if} \quad Q^*_{C-E} \leq N:C_F$$

where
$Q^*_{C-E} = K_C\,(N:C_Z)$, or the critical food nutrient (nitrogen to carbon) ratio below which fish production would be partially limited by nitrogen.
$N:C_F$ = nitrogen to carbon mass ratio in food
K = gross growth efficiency of fish
$N:C_Z$ = nitrogen to carbon ratio in fish

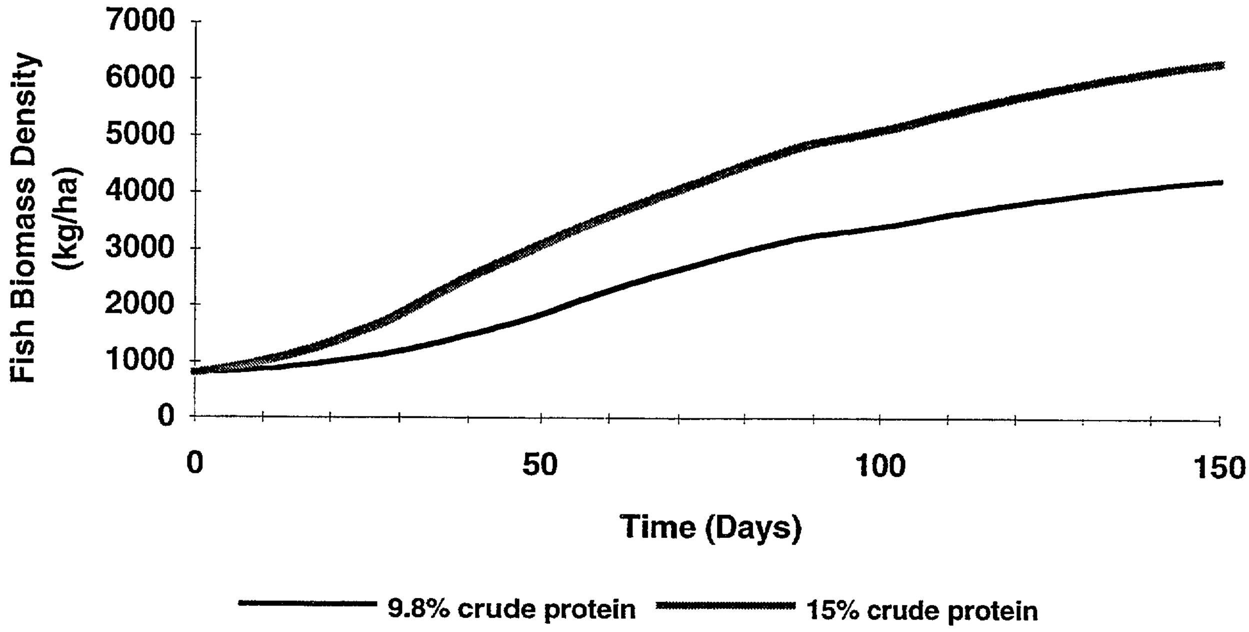

Figure 2. Fish biomass production at two levels of crude protein in artificial feed simulated using a modified bioenergetic fish growth model.

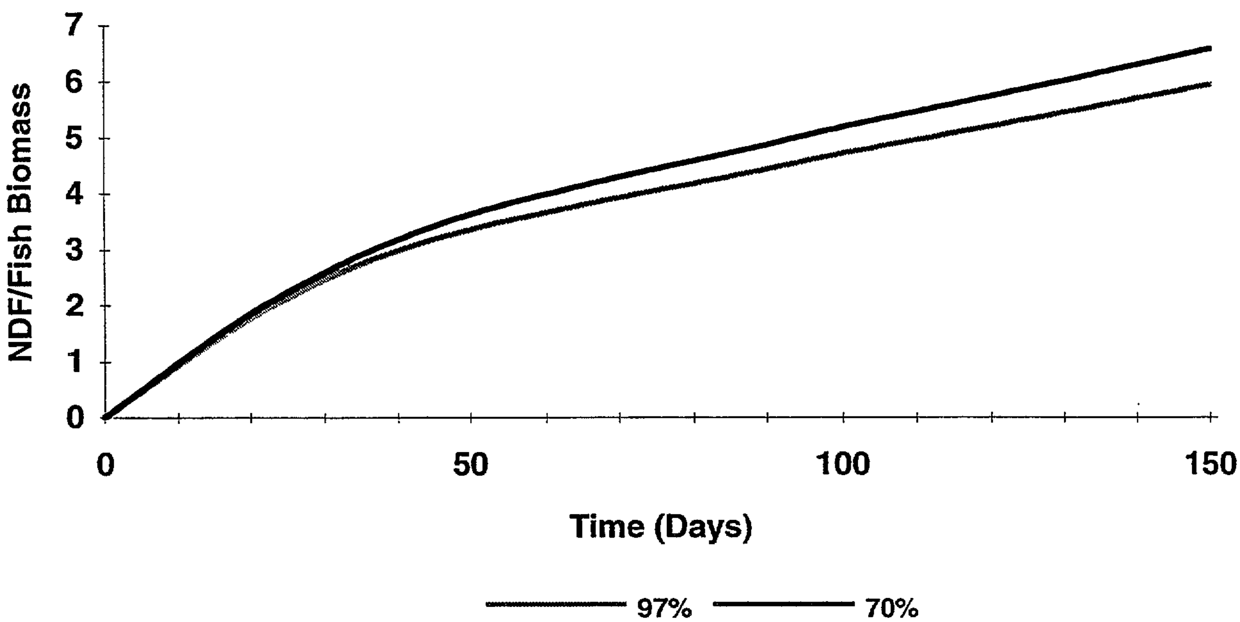

Figure 3. Simulated non digested feed (NDF) production using two different digestibility coefficients. NDF is normalized for fish biomass by dividing NDF with fish biomass.

Results and Discussion

Modifications to the fish growth model are summarized in Equation 1 and 3. To demonstrate the importance of incorporating feed quality and separate digestibility coefficients for artificial feed and phytoplankton, the model was run for a period of 150 days. Two model runs were made to demonstrate the problems that may arise in simulating fish biomass when feed quality is not taken into account. The runs were made with artificial feed crude protein contents set at 9.8 and 15% (Figure 2). Digestibility was constant at 97% for both protein levels. The values for crude protein and digestibility correspond to those reported for corn grain (Stickney, 1994). The higher protein content resulted in higher growth rates and larger fish.

A second simulation of non digested feed production was run using constant crude protein content in the feed and two levels of digestibility coefficient: 70 and 97% (Figure 3). The non digested feed was normalized for fish biomass. The differences in production of non digested feed at the two digestibility coefficients demonstrate that potential errors could be incorporated in organic matter /nitrogen pools and fluxes when an average coefficient is used for feed items. Since one of the objectives of the model is to analyze and predict nitrogen and organic matter outputs from an aquaculture pond, accurate estimation of organic matter is necessary.

Anticipated Benefits

The model being developed will provide results that improve our understanding of the relationship between organic matter inputs and sediment nitrogen retention. The results will help farmers identify feed and fertilizer types that promote the development of useful pond sediments. In intensive systems, the results will help in the management of nitrogen, where sediment nitrogen retention could reduce ammonia in the water column and nitrate loss to surface and groundwaters.

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Stochastic Modeling of Temperature and Dissolved Oxygen in Stratified Fish Ponds

Work Plan 7, DAST Study 2; Work Plan 8, DAST Study 1

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Introduction

Research on water quality modeling in aquaculture ponds has involved, for the most part, the development of deterministic models. In a deterministic model, the outcome of the model is always the same for a given set of input parameters. There are very few examples of models used in aquaculture in which stochastic variables, equations, or parameters are considered. Sadeh et al. (1986) used random water quality parameters in their study of economic profitability of shrimp production in ponds. In their model, water temperature was used as a determinant factor of shrimp growth rate. In turn, water temperature was determined from air temperature using a linear equation. The probabilities of temperature occurrences were estimated from records spanning more than 40 years. The effects of light and phytoplankton concentration on shrimp growth were not considered in their model.

Recent work undertaken by the UC Davis DAST has resulted in a first version of a water quality and fish growth model using stochastically generated weather parameters (solar radiation, wind speed and wind direction), and that model is described in this report. The water quality component of the model is based on a deterministic model of stratified ponds developed by Losordo (1988) and modified by Culberson (1993). The temperature model is based on an energy balance for the water column (Losordo, 1988). Dissolved oxygen (DO) is the primary variable considered in the water quality model, and is determined from mass balance calculations considering oxygen production, consumption, and transfer terms. Weather parameters (solar irradiance, air temperature, wind speed and direction) are critical input variables in modeling both water temperature and DO. Therefore, weather parameters for a stochastic pond model need to be obtained from some statistical treatment of existing records for a site.

Numerous papers have been published on modeling weather parameters. Program simulations have been developed generally using the probability distributions generated from measured values (Amato, et al., 1986). To obtain an accurate distribution, a long-term, complete data set is required. Unfortunately, large, reliable data sets are rarely available for prospective aquaculture sites, especially in rural areas, and some of the work undertaken in this project has been targeted at finding ways to make optimum use of limited weather data sets.

Model Structure

The combined water quality/fish growth model consists of four parts : 1. weather parameter generation; 2. temperature simulation; 3. DO simulation; 4. fish growth simulation. Initial versions of the first two parts of the model have been described by Santos Neto and Piedrahita (1994).

Weather parameter generation

The simulation of hourly values for solar radiation is carried out in two steps. A total solar radiation value for a given date is generated in the first step. The distribution of the total daily solar radiation over a day is obtained in the second step, and hourly values are estimated (Santos Neto and Piedrahita, 1994).

Total daily solar radiation for a given date is obtained from (Amato et al., 1986):

$$H_i = \mu(i) + \sigma(i) \cdot \hat{\chi}(i,j)$$

for i=1, 2,...n; j=1, 2, ..., m (i = day, j = year)

where, (1)

H_i = generated total daily solar radiation for day i

$\mu(i)$= mean daily solar radiation value on the ith day for m years

$\sigma(i)$ = standard deviation for solar radiation on day i

$\hat{\chi}(i,j)$ = estimated residual value, which is given by

$$\hat{\chi}(i,j) = \rho \cdot \hat{\chi}(i-1,j) + (1-\rho^2)^{0.5} \cdot \varepsilon(i,j) \quad (2)$$

where,
ρ = autocorrelation coefficient of lag one between two consecutive days

$\varepsilon(i,j)$ = random term generated for each day from a normal distribution with a mean of zero and a standard deviation of one

Values for the terms in Equations 1 and 2 are obtained from measured solar radiation values in the CRSP data base. The daily mean values ($\mu(i)$), daily standard deviations ($\sigma(i)$), and the autocorrelation coefficient (ρ) are calculated for a given solar radiation data set using a program called SolarRad written in BASIC. The values obtained from SolarRad are then input to the combined water quality/fish growth program which is written in Stella™. The estimated residual term and the total solar radiation for a given day (Equations 2 and 1, respectively) are calculated in the combined program for each day for which a simulation is run. Hourly solar radiation values can be obtained from

$$RandomHourly_{i,t} = H_i \cdot \frac{SolarHourly_t}{SolarMean} \quad (3)$$

where,

$RandomHourly_{i,t}$ = generated random hourly solar radiation value at time t, on day i,

$SolarHourly_t$= estimated hourly solar radiation at time t, for the "typical" day

$SolarMean$ = average integrated solar radiation received by the pond on the "typical" day.

$SolarHourly_t$ and $SolarMean$ are calculated from measurements of solar radiation obtained during the diel sampling events (Santos Neto and Piedrahita, 1994).

Wind speed values are determined by sampling from a normal distribution with mean and standard deviation values obtained from historical data. The procedure used is different from that used for solar radiation due to the limited wind data available. A simple stochastic equation is used in which a stochastic perturbation is added to the mean value for a given time of day (Santos Neto and Piedrahita, 1994; Swartzman and Kaluzny, 1987):

$$W_t = Wmean_t + \varepsilon_t(\sigma_w) \quad (4)$$

where,

W_t = generated value for wind speed at time t.

$Wmean_t$ = mean value for wind speed at time t, from historical records

σ_w = standard deviation for wind speed at time t, from historical records

ε_t = random term generated by sampling from a normal distribution with a mean of zero and a standard deviation of one.

Wind direction is generated from a skewed normal distribution. The standard deviation, mean value, and skewness are calculated from historical records for a given site. The random component of wind direction is once again generated by sampling from a normal distribution with a mean of zero and a standard deviation of one:

$$W = \frac{\pi}{180} \cdot (\sigma \cdot \frac{2}{s} \cdot ((1 + \frac{s}{6} \cdot (\varepsilon - (\frac{s}{6})))^3 - 1) + \mu) \quad (5)$$

where,
W = generated wind direction, (radians)
σ = standard deviation (degrees) calculated from historical records
ε = random term generated by sampling from a normal distribution with a mean of zero and a standard deviation of one
μ = mean wind direction (degrees) calculated from historical records
s = skewness coefficient calculated from historical records

Temperature model

Water temperature is calculated from an energy balance as described by Losordo (1988) and by Culberson (1993). The main heat sources in a pond are solar irradiance and atmospheric radiation. Incident solar irradiance is obtained from generated solar radiation values as described above. Atmospheric radiation is determined by air temperature. The attenuation of solar irradiance with depth in the water column is estimated by using a bulk light extinction coefficient determined as a function of Secchi disk depth. Since the daily Secchi disk depth was not available in the CRSP data base, a site-specific regression equation between Secchi disk and chlorophyll-a concentration is used in the model. Energy exchange between layers in the pond water column is due to diffusion and convection (Losordo, 1988). Diffusion is estimated primarily as a function of wind speed, wind direction, and fetch (Losordo, 1988; Culberson, 1993).

Dissolved Oxygen model

Dissolved oxygen concentrations are determined from mass balance calculations in which photosynthesis constitutes the main source of oxygen. Oxygen sinks include respiration by phytoplankton, fish, benthic organisms, etc. Oxygen transfer with the atmosphere may constitute a source or a sink, depending on whether the oxygen concentration in the surface layer of the pond is below or above saturation. Calculation of oxygen production and consumption rates requires some estimate of phytoplankton concentration. In previous models Losordo (1988) and Culberson (1994) used measured chlorophyll-a values, but those are not available on a daily basis as needed for simulations covering many consecutive days. Therefore, Culberson's model was modified by adding a mass balance term for chlorophyll-a, and introducing a term relating phytoplankton carbon to chlorophyll-a concentration (carbon:chlorophyll-a ratio, or CCHL). CCHL is highly variable, and values are reported between 10 and 1000 (Steele, 1962). An estimate of CCHL can be obtained from (Lee et al., 1991 a, b):

$$CCHL = \frac{24 \cdot \alpha \cdot I_{\max}}{\mu_{\max} g(t) e} \qquad (6)$$

where,

$CCHL$ = ratio of phytoplankton carbon to chlorophyll a, $\left(\frac{mgC}{mgChla}\right)$

α = slope of photosynthesis rate to light intensity (P-I) curve, $\left(\frac{mgC}{mgChla \cdot \frac{w}{m^2}}\right)$

$I_{\max}$ = maximum light intensity, $\left(\frac{w}{m^2}\right)$ (7)

$\mu_{\max}$ = maximum phytoplankton growth rate, $\left(\frac{mgO_2}{mgC \cdot hr}\right)$

$g(t)$ = temperature dependence, $1.08^{(t-20)}$

e = constant, 2.718

In the model, α and $\mu_{\max}$ are inferred from a previous deterministic model (Culberson, 1993). CCHL is depended on the maximum light intensity $I_{\max}$ and water temperature. In the present model, $I_{\max}$ is determined as the weighted average of the light intensity for the previous three days (Lee et al. 1991b):

$$I_{\max} = 0.7 I_{\max 1} + 0.2 I_{\max 2} + 0.1 I_{\max 3}$$

where $I_{\max}$, $I_{\max 2}$, and $I_{\max 3}$ are the lightest intensities one, two, and three days prior to the current day

Fish growth rate

The fish growth model was adapted from the model developed by the OSU-DAST (Bolte et al., 1994). The adaptation was carried out as part of work carried out by the UC Davis DAST, on modeling of integrated aquaculture-agriculture systems, and is described by Jamu and Piedrahita (Aquaculture Pond Modeling for the Analysis of Integrated Aquaculture/ Agriculture Systems, Work Plan 8, Study 2 in this volume).

Results and Discussion

A period of 83 days was simulated, from Julian day 40 to 123, with a time step of 0.0625 hours. The length of the simulation was limited by the internal structure of the modeling software (16 bit addresses, or a maximum of 32,768 steps), and was independent of hardware (same limitation on Macintosh™ and Windows™ machines). Simulation results (maximum, minimum, and average values) are obtained after running the model 50 times using stochastically generated weather parameters as described above, and are shown in Figures 1 through 15. The data used for model execution were collected at the CRSP site in Thailand, and correspond to the data set used in the development of previous models by the UC Davis DAST (Santos Neto and Piedrahita, 1994; Culberson, 1993). Simulated water temperatures of the three layers are shown in Figures 1 through 3. Measured values available for the 83 day time period lie within the range of temperatures defined in the simulations with two exceptions, one on day 82, and one on day 110 (0.5, and 0.3 °C higher than the maximum temperatures simulated for the corresponding times). Simulation results for the middle and bottom layers lie further from the mean values, and stray beyond the range of temperatures estimated from the simulations for the bottom temperature for days 40 and 110 (Figures 5 and 6).

Dissolved oxygen fluctuations were more pronounced for the surface layer than for the middle and bottom layers (Figures 7-9). The probability of the pond dissolved oxygen concentration dropping below 2 mg/L was most evident during the second half of the simulation period, where the minimum DO calculated often was under 2 mg/L during at least part of the day in all three layers (Figures 7-9). The minimum DO calculated over the 50 simulation runs reached 0 mg/L for all three layers on days 102 and 103 (Figures 7-9). Comparing the simulated and measured DO values for the three layers shows better agreement for the surface and middle layer than for the bottom layer (Figures 10-12). The large fluctuations of temperature and DO are caused primarily by the large changes of the stochastically generated solar radiation values (Figure 13). The maximum solar radiation intensity generated, I_{max} ranged from 500 to 2800 µmol/m^2/s, with the measured values being lower than the mean of the predictions for most days (Figure 13).

Chlorophyll-*a* concentration rises throughout the simulation period (Figure 14), but no data are available in this particular data set to compare to the simulated values. However, chlorophyll-a concentrations are often observed to rise during a growing season. Fish biomass is being overestimated in the current version of the model (Figure 15), and revisions to the fish growth estimation as a function of feed quantity and quality, and of water quality parameters will be necessary to improve the accuracy of the predictions.

Anticipated Benefits

The results presented in this report are for the first version of a model of water quality and fish growth in fish ponds using stochastic weather inputs. The results show the power and usefulness of using a stochastic approach to simulate pond production. By being able to generate a range of possible water quality and fish yield outcomes for a site and for a particular pond management strategy, the modeler will be able to identify risks associated with a given operation and make more informed decisions on site selection and on pond management. Improvements are needed in the model, especially in the dissolved oxygen and fish growth simulations.

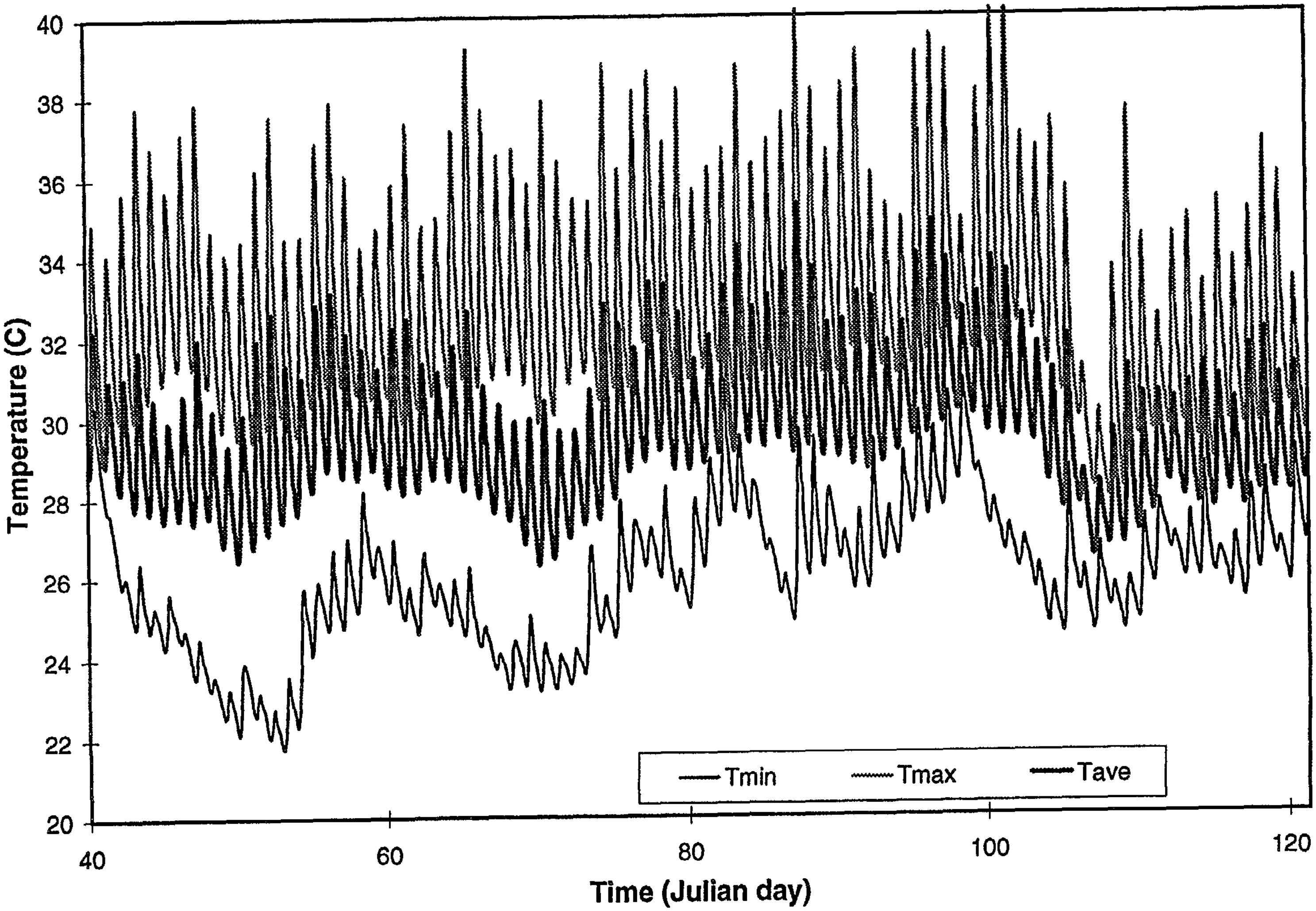

Figure 1. Temperature predictions for the surface layer of a stratified pond after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean temperature obtained at each hour of the simulation are shown.

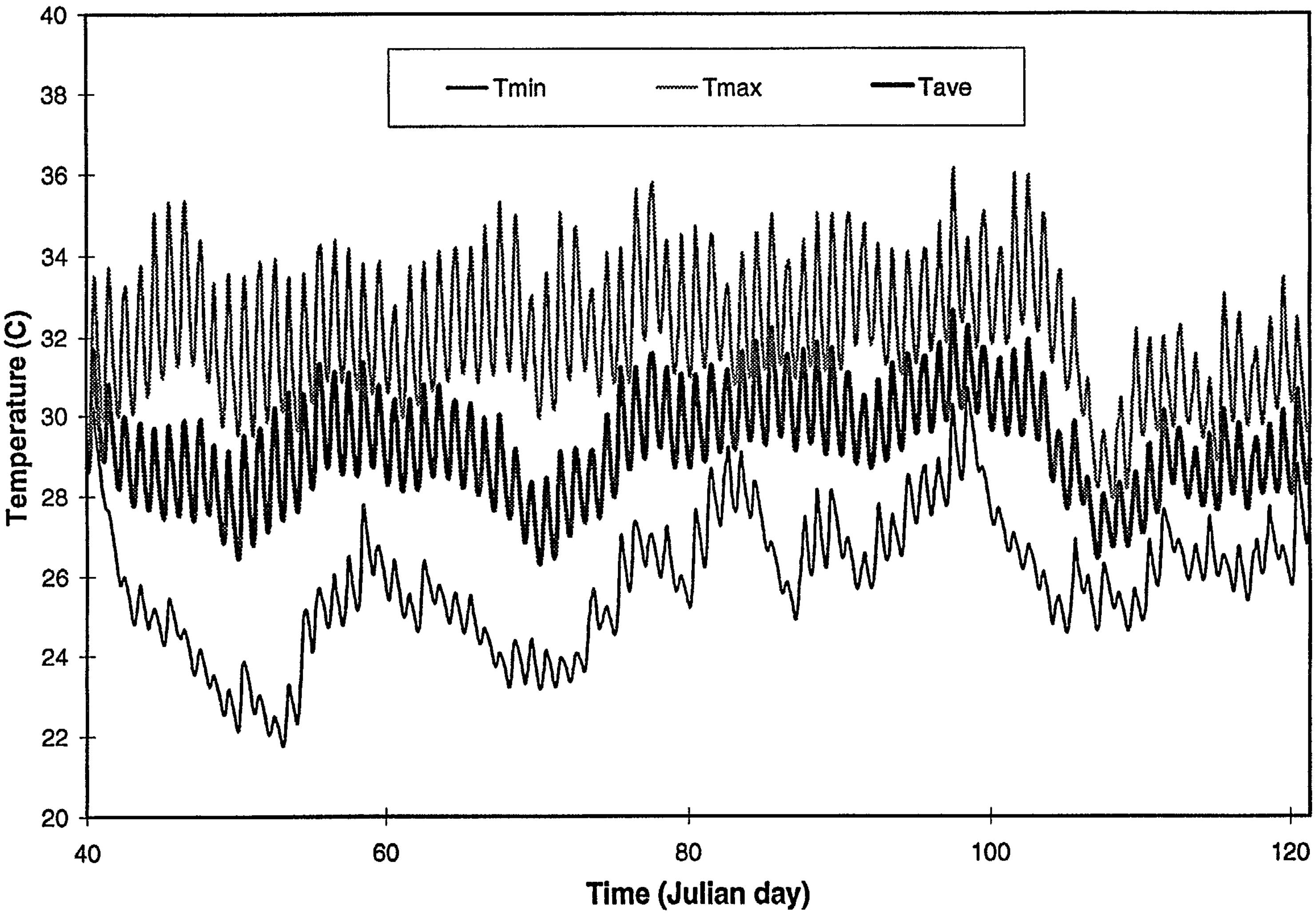

Figure 2. Temperature predictions for the middle layer of a stratified pond after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean temperature obtained at each hour of the simulation are shown.

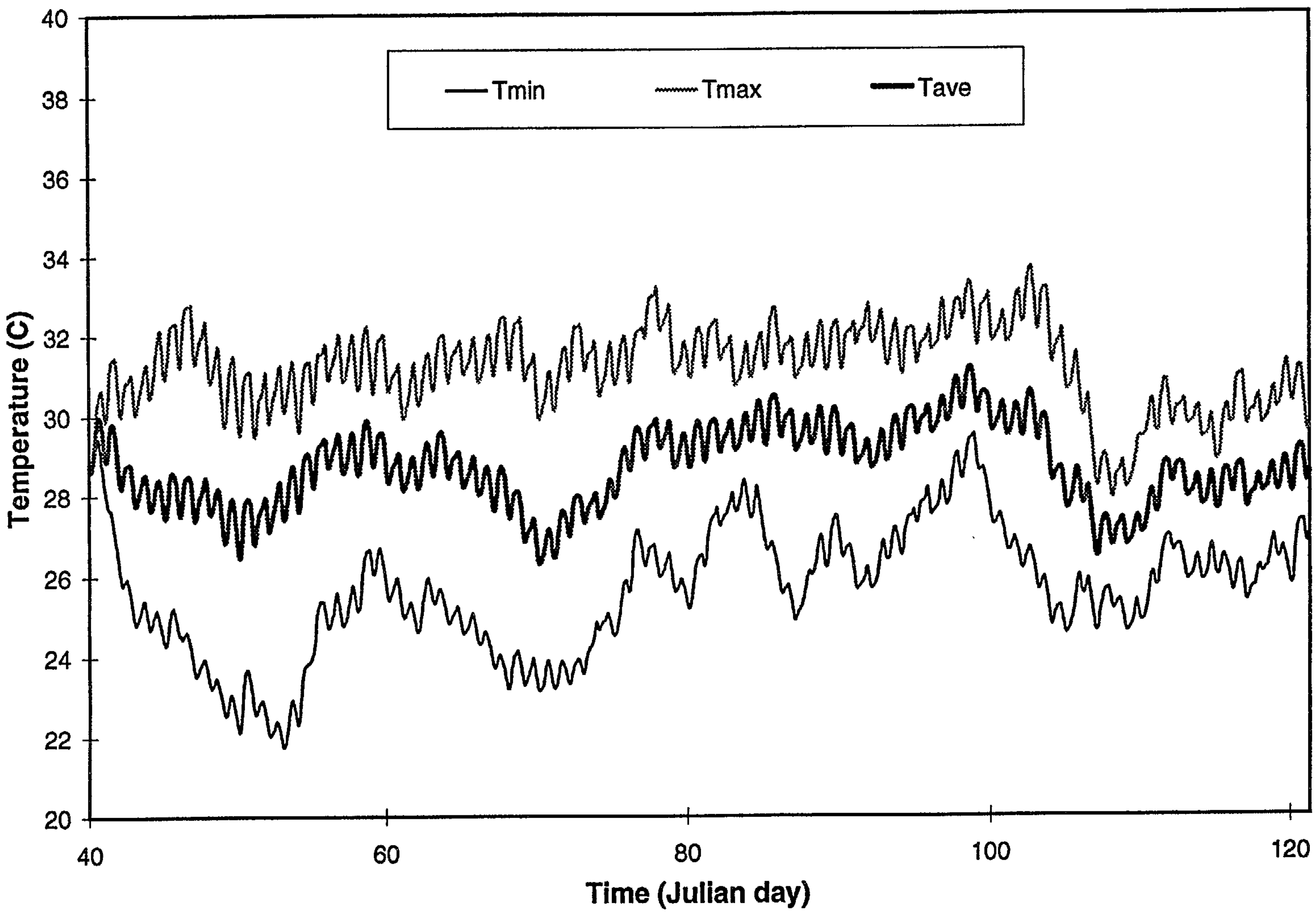

Figure 3. Temperature predictions for the bottom layer of a stratified pond after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean temperature obtained at each hour of the simulation are shown.

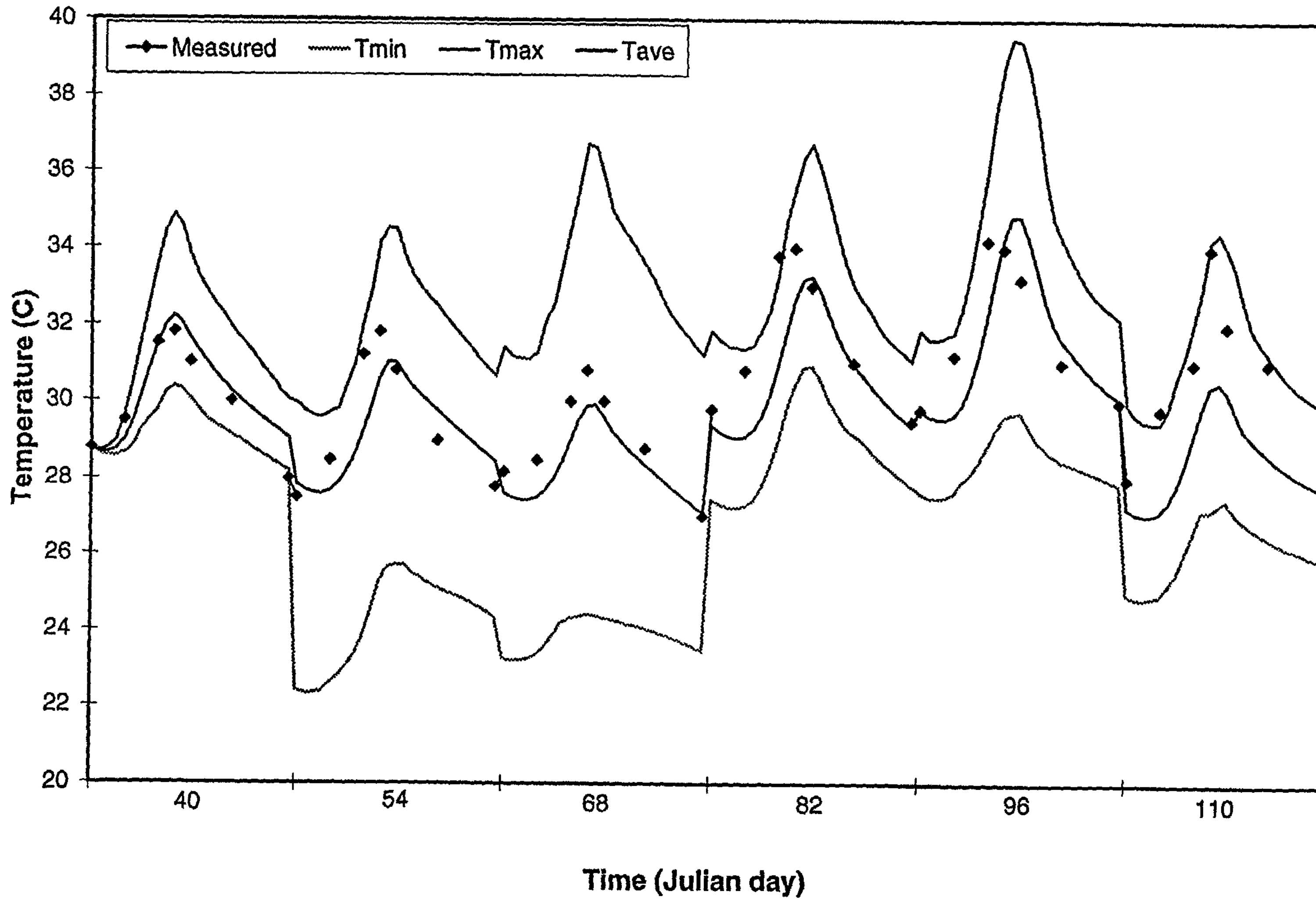

Figure 4. Comparison of measured and predicted temperatures for the surface layer. The days shown are those for which measured values were available.

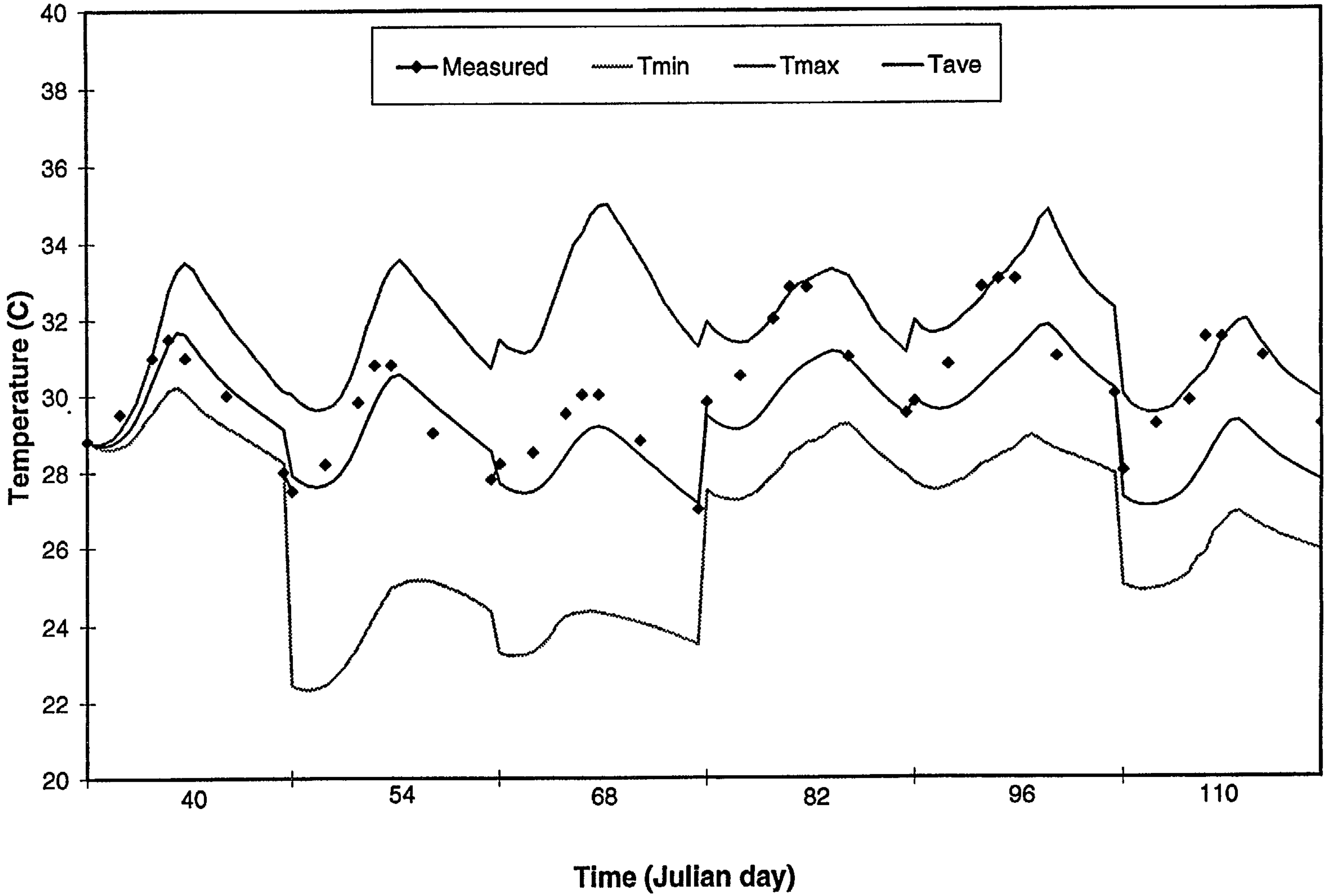

Figure 5. Comparison of measured and predicted temperatures for the middle layer. The days shown are those for which measured values were available.

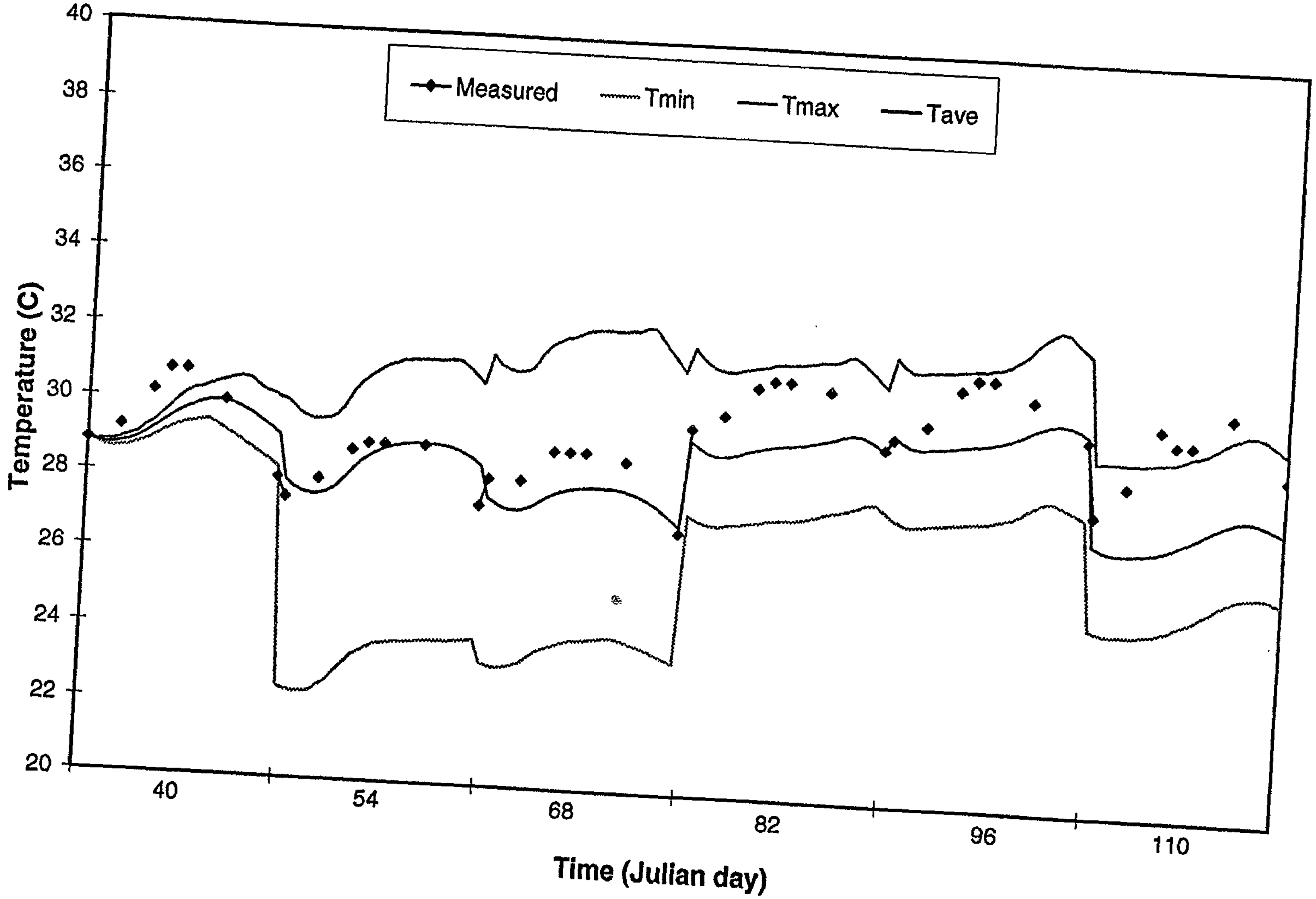

Figure 6. Comparison of measured and predicted temperatures for the bottom layer. The days shown are those for which measured values were available.

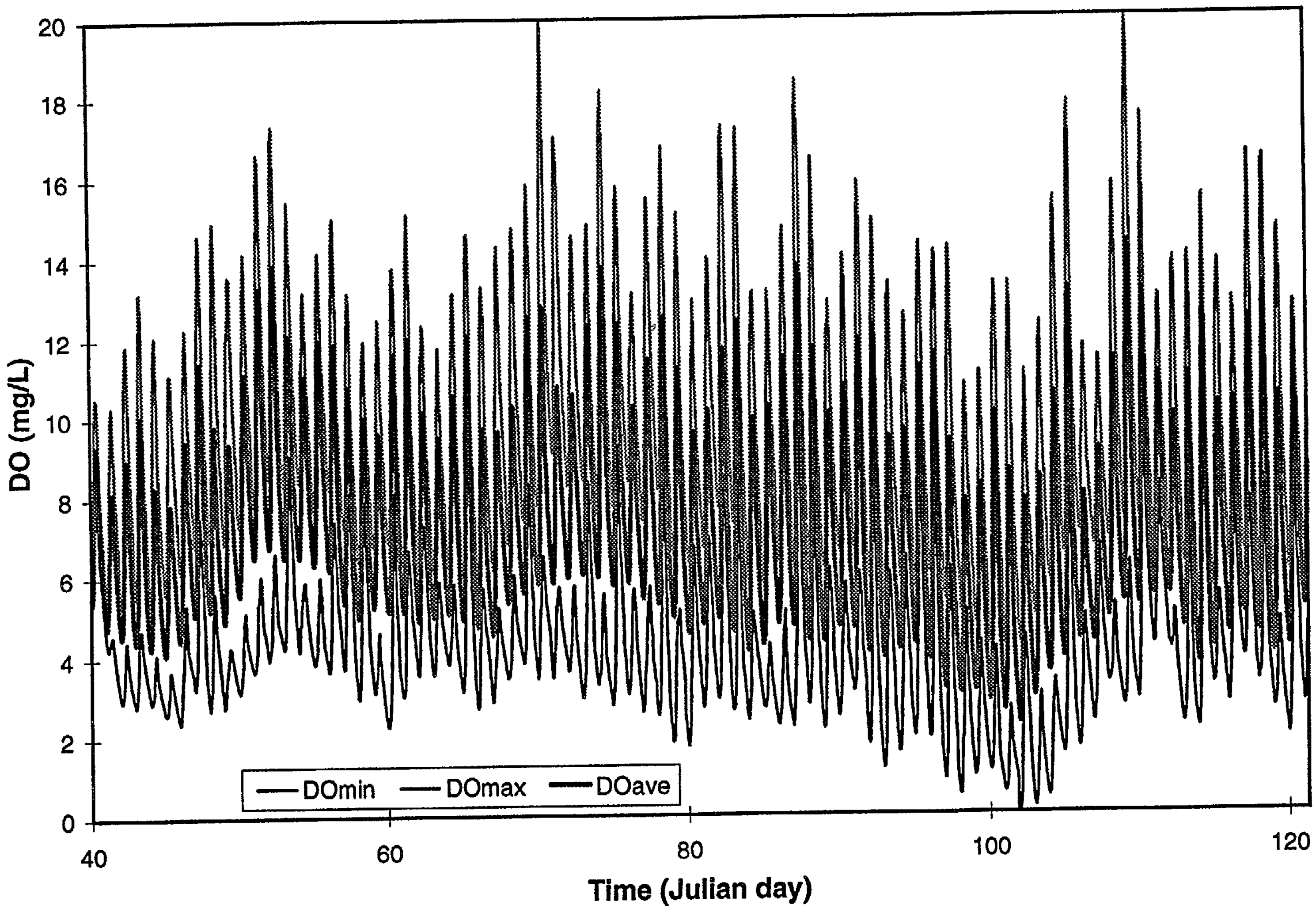

Figure 7. Dissolved oxygen predictions for the surface layer of a stratified pond after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean DO obtained at each hour of the simulation are shown.

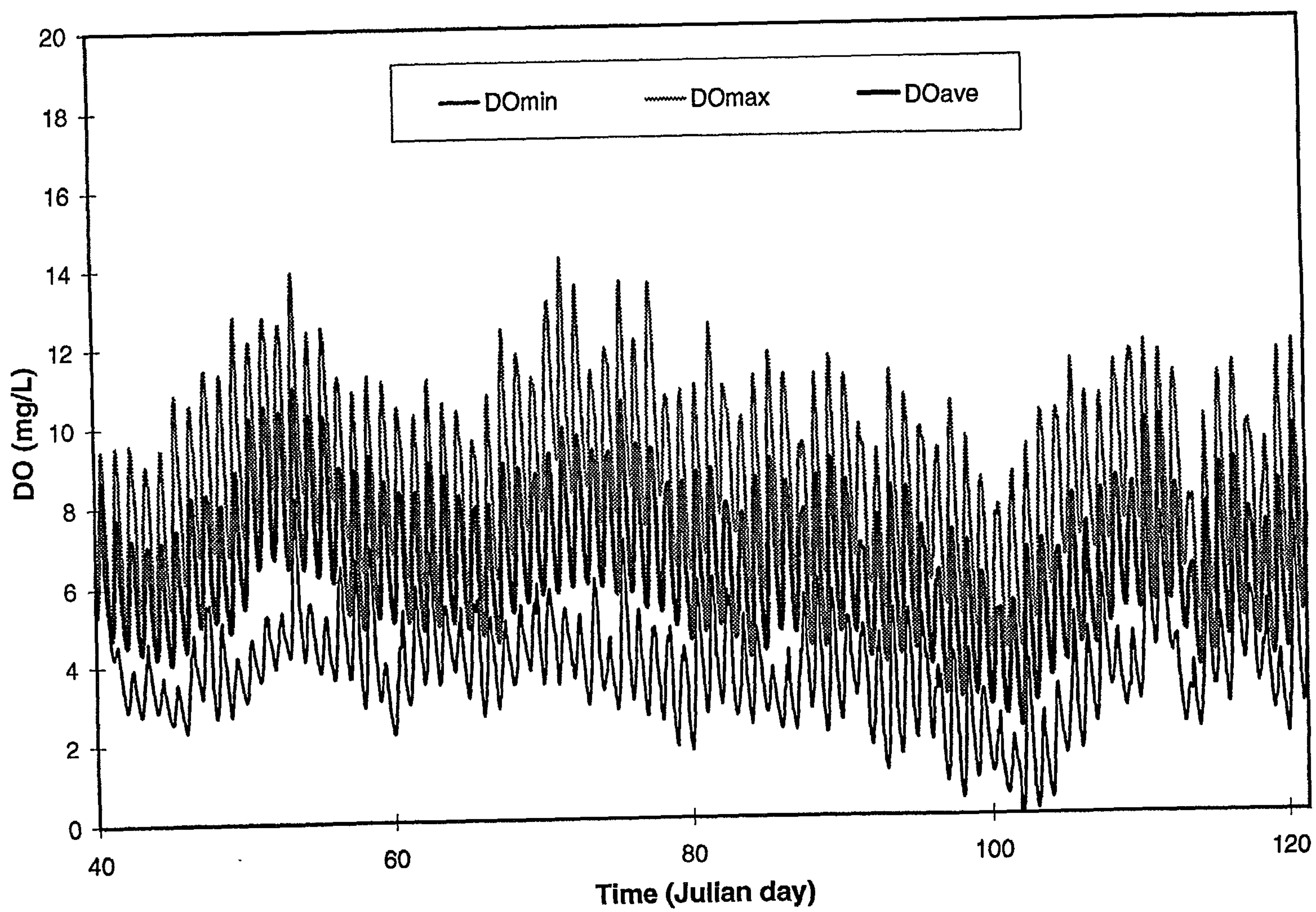

Figure 8. Dissolved oxygen predictions for the middle layer of a stratified pond after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean DO obtained at each hour of the simulation are shown.

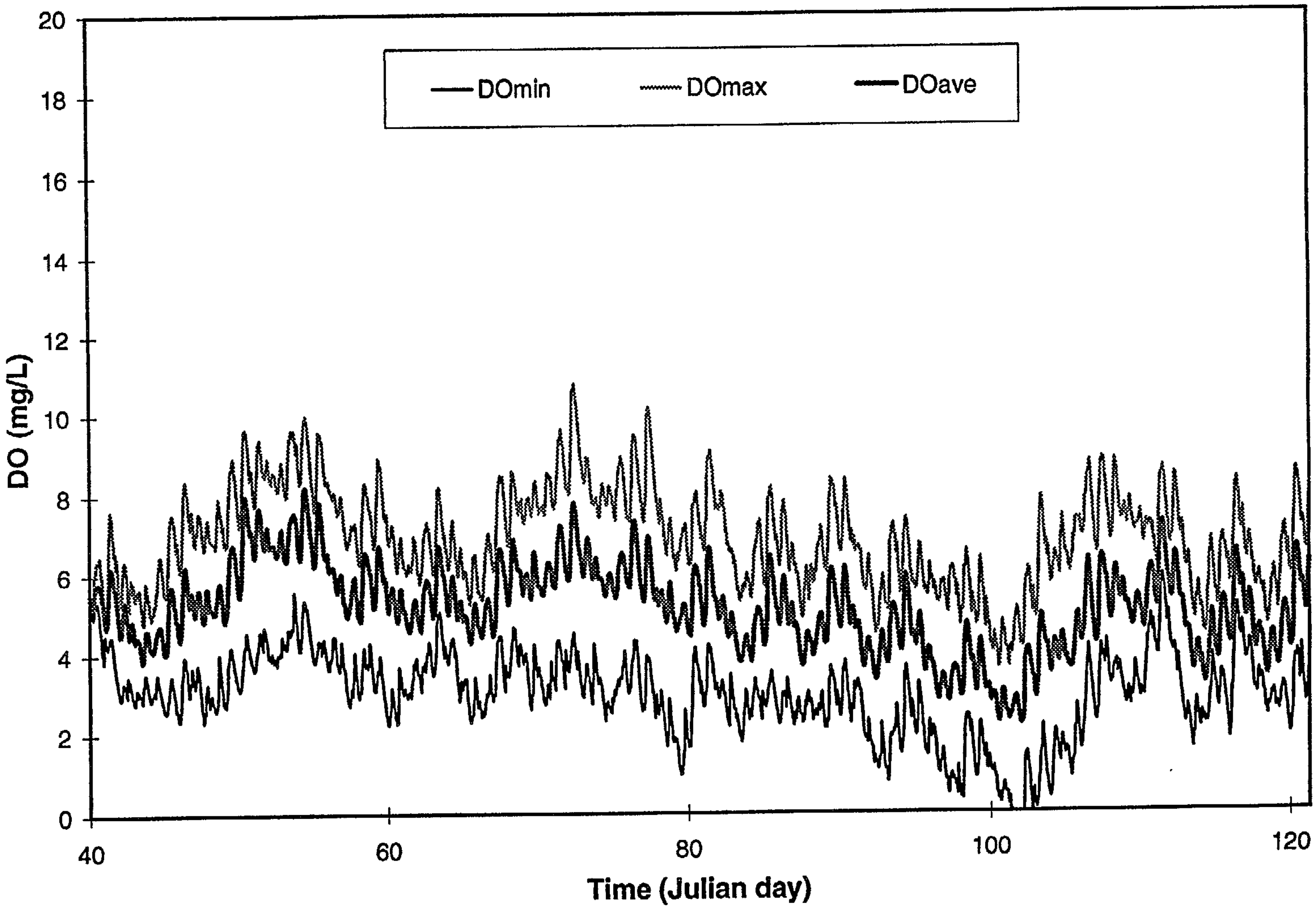

Figure 9. Dissolved oxygen predictions for the bottom layer of a stratified pond after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean DO obtained at each hour of the simulation are shown.

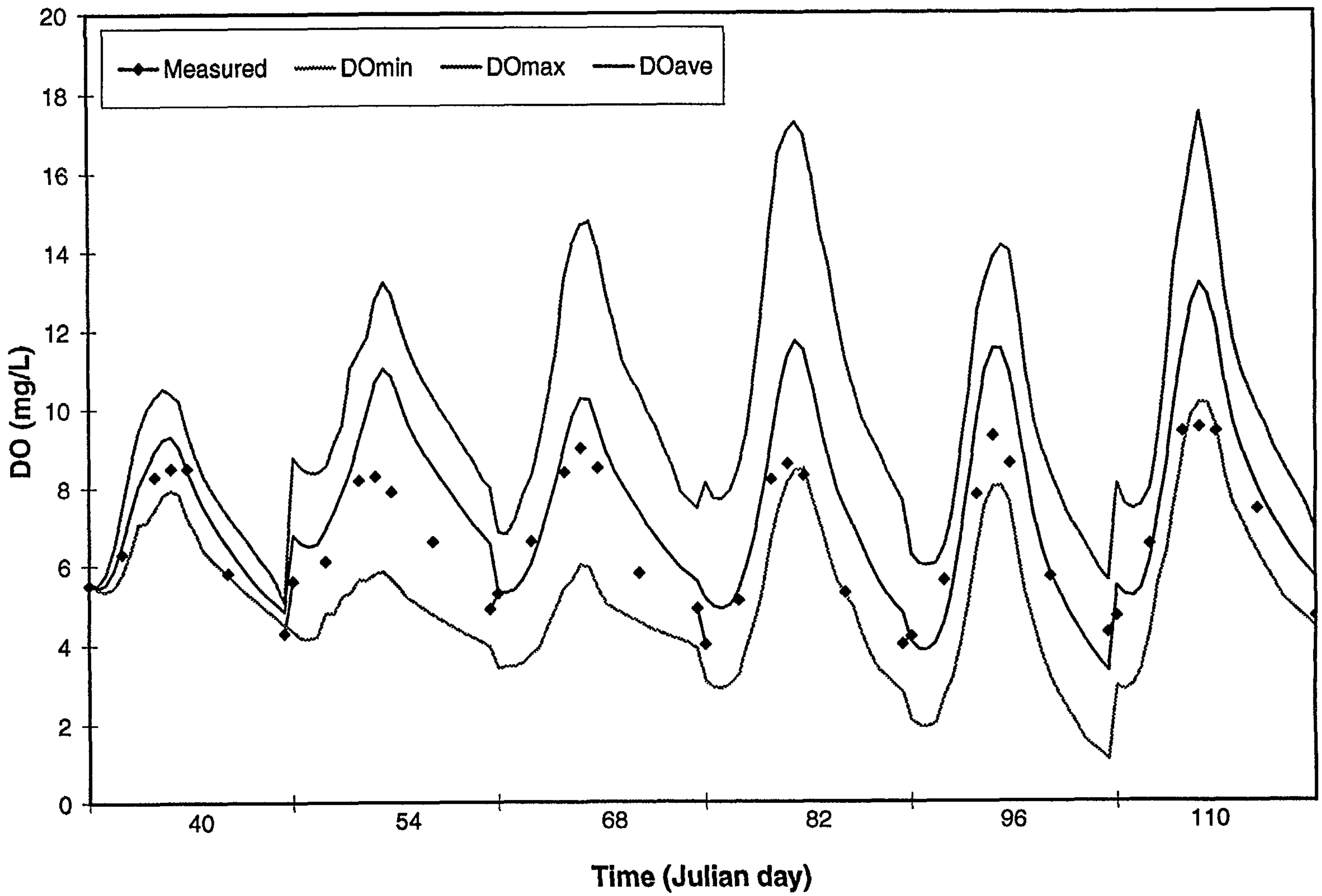

Figure 10. Comparison of measured and predicted DO for the surface layer. The days shown are those for which measured values were available.

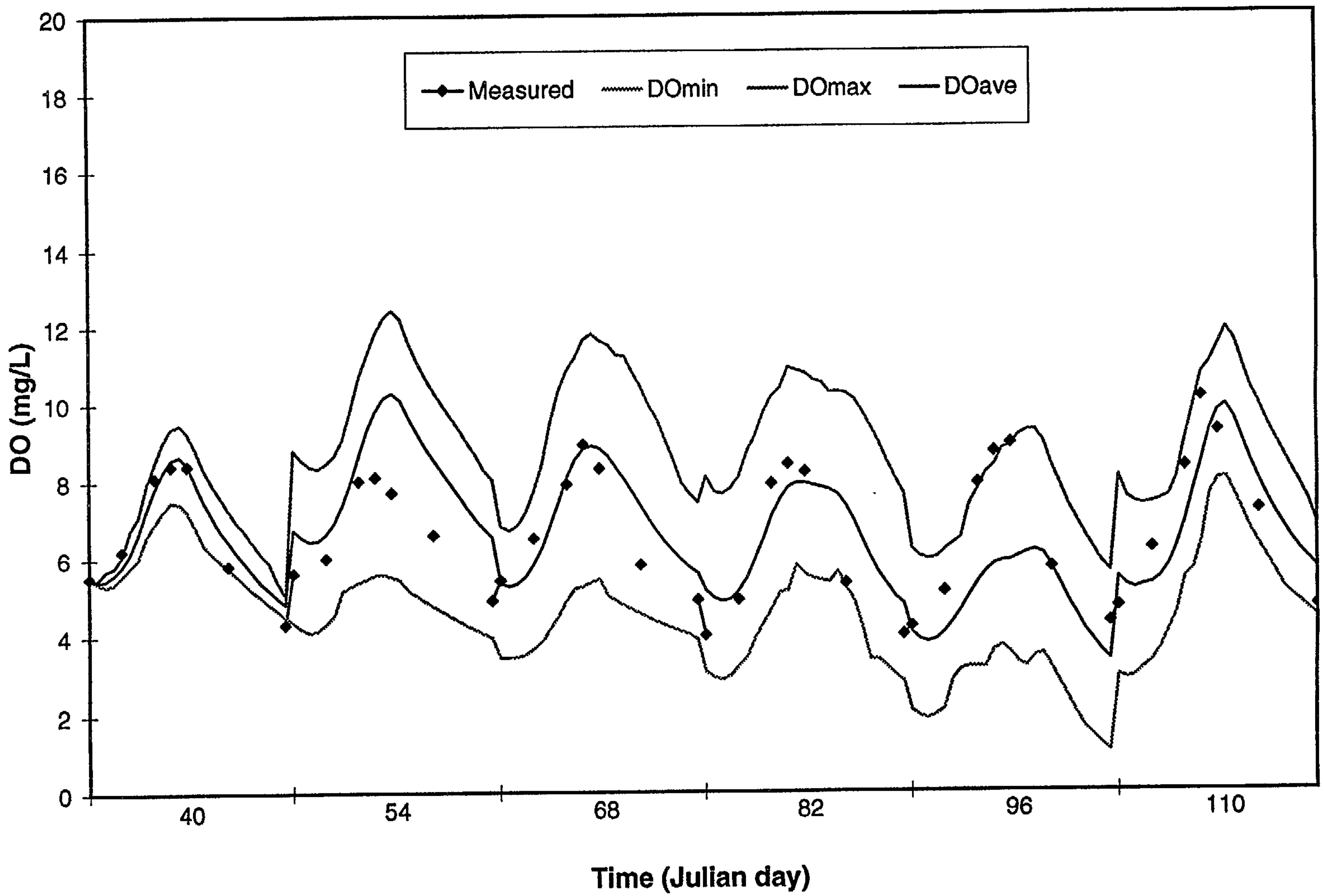

Figure 11. Comparison of measured and predicted DO for the middle layer. The days shown are those for which measured values were available.

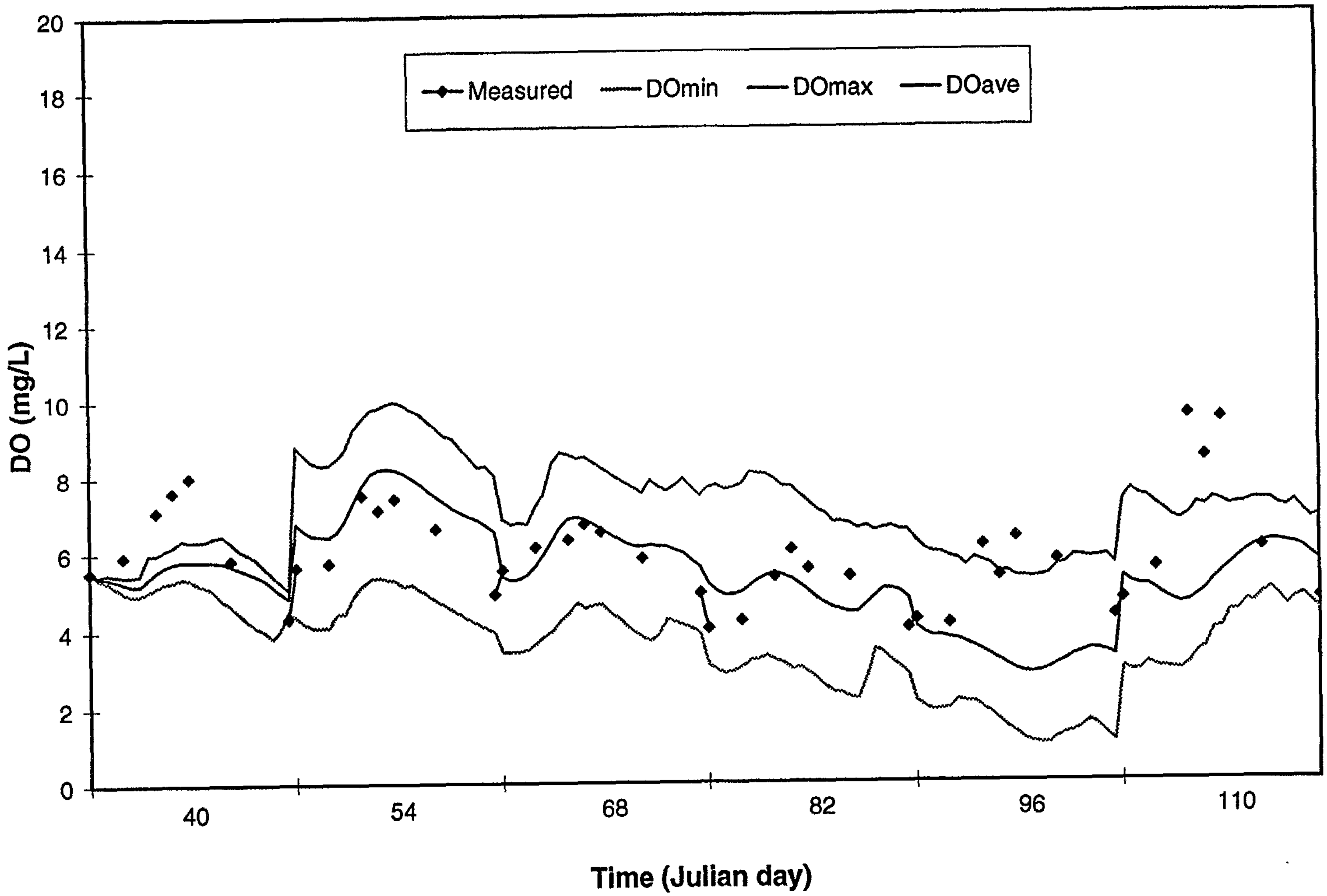

Figure 12. Comparison of measured and predicted DO for the bottom layer. The days shown are those for which measured values were available.

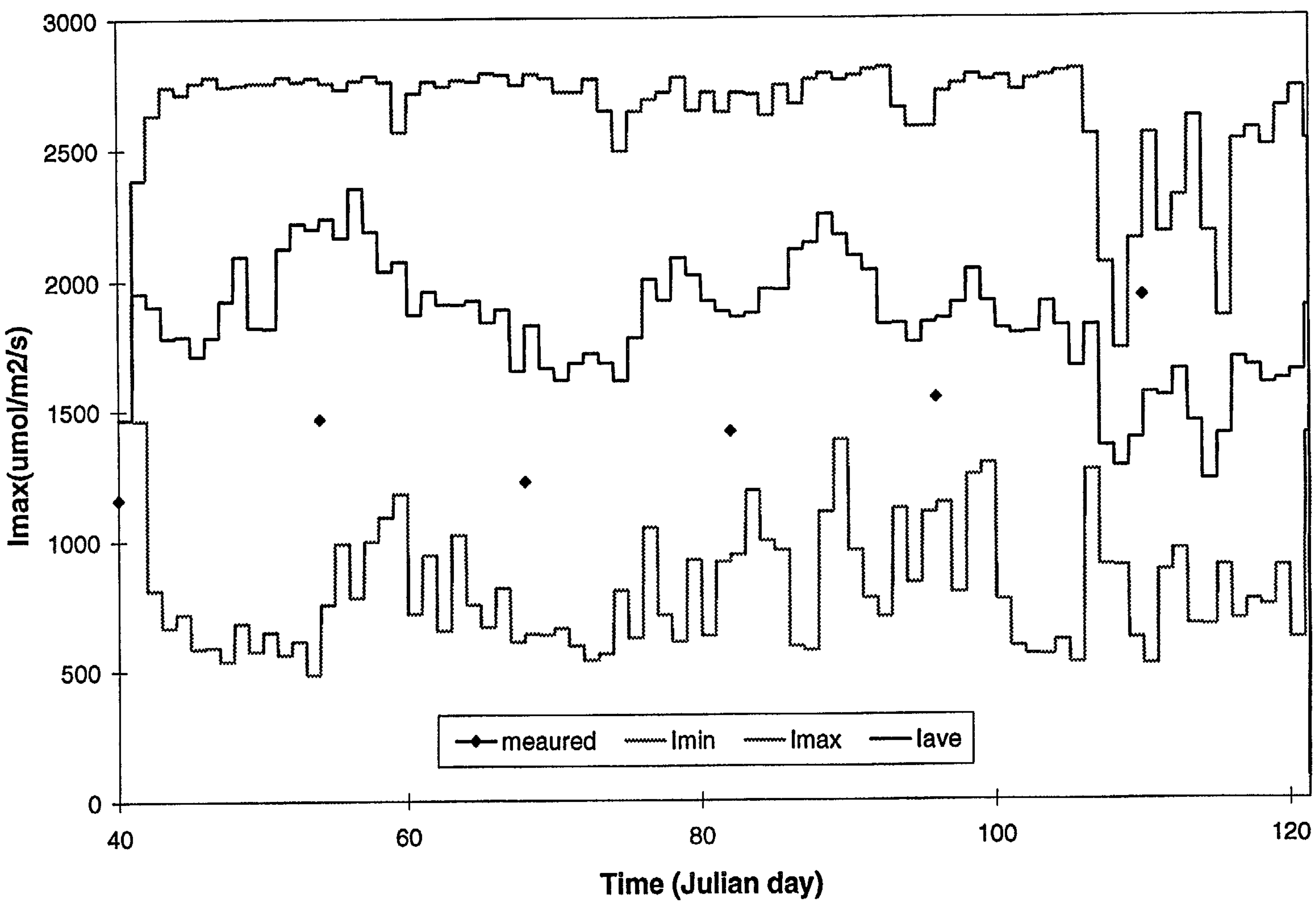

Figure 13. Maximum solar radiation intensity (I_{max}) obtained after 50 simulations compared to measured values available.

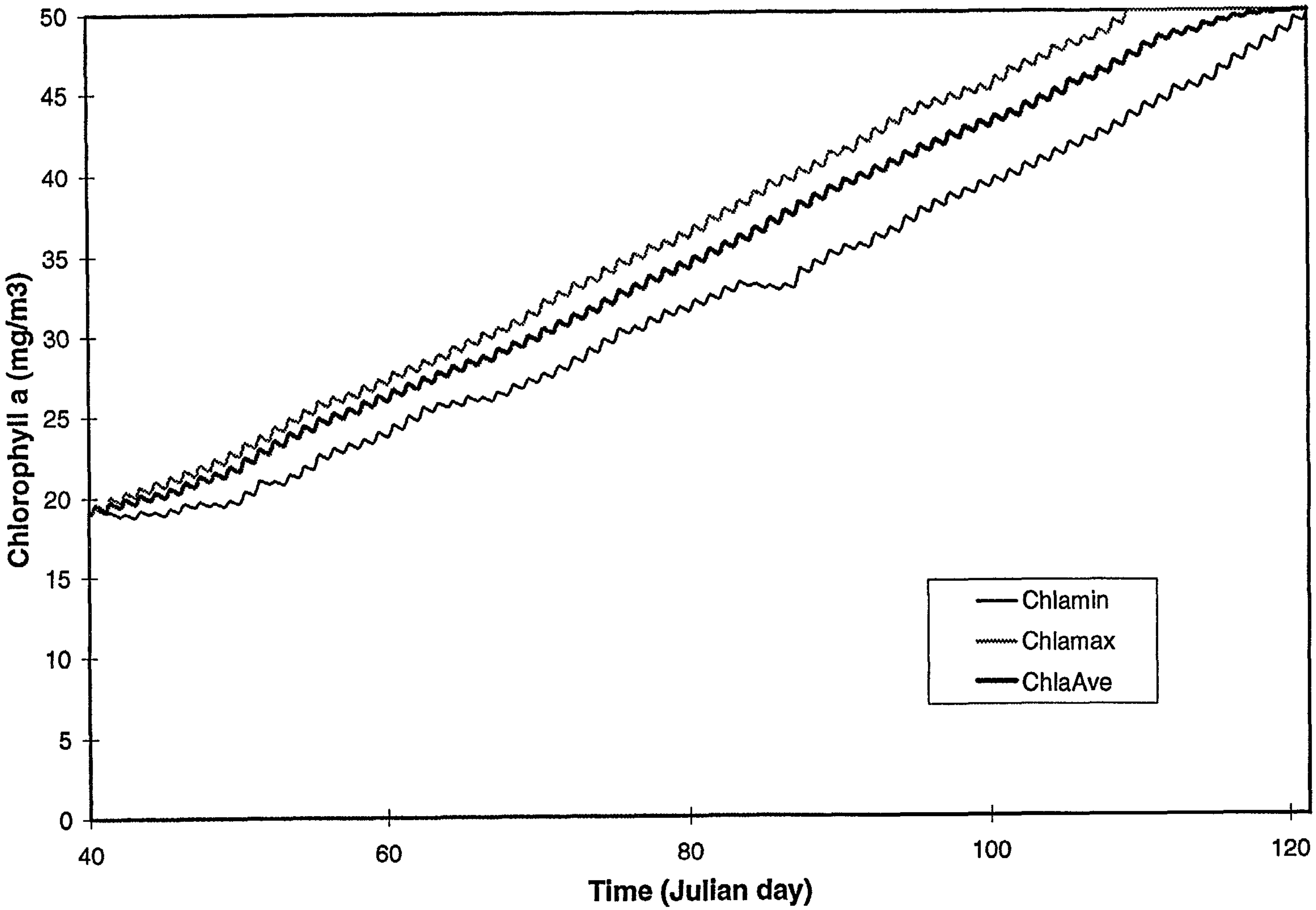

Figure 14. Chlorophyll-*a* predictions after 50 runs, and over an 83 day simulation. The maximum, minimum, and mean values obtained at each hour of the simulation are shown.

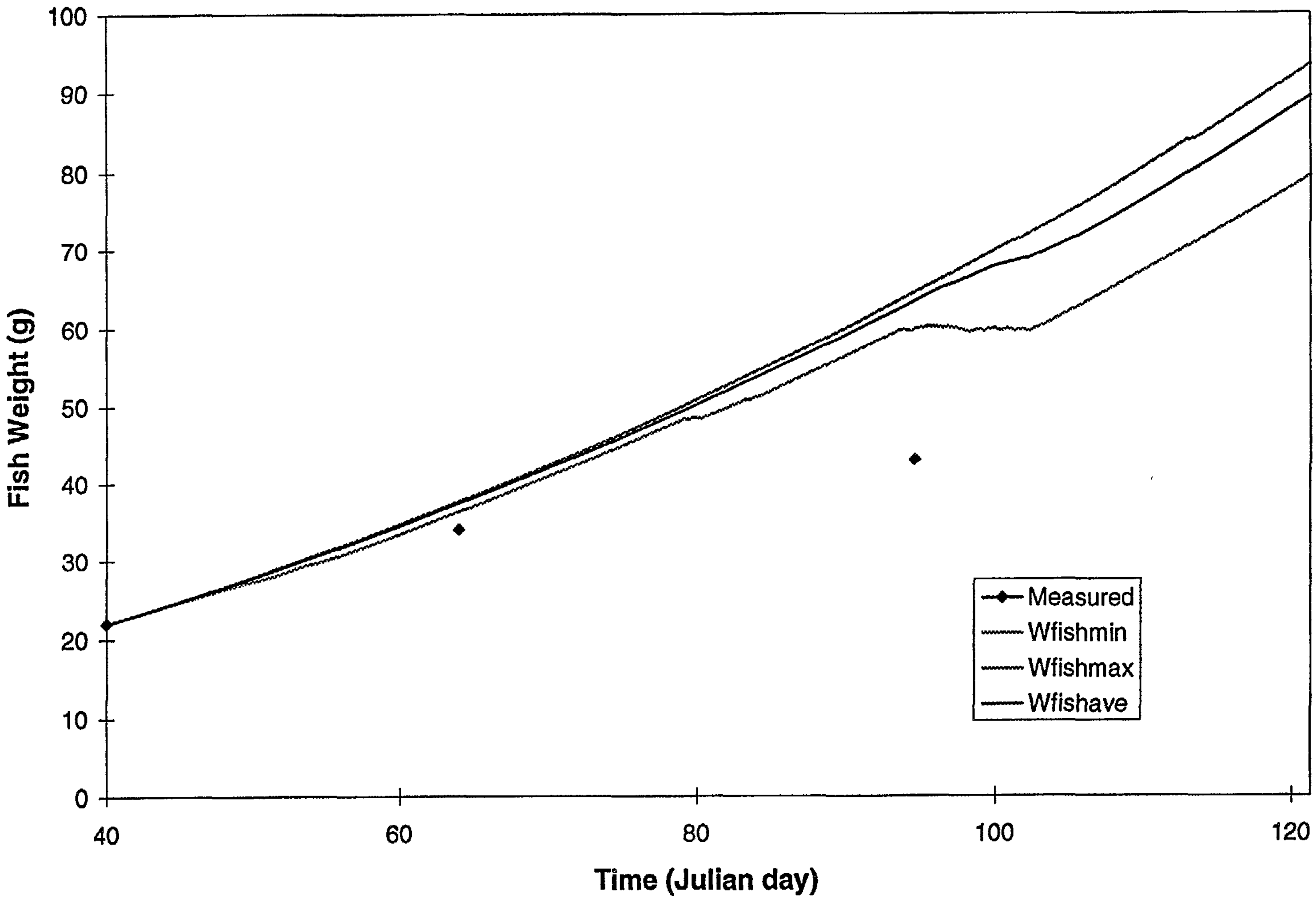

Figure 15. Individual fish mass predicted after 50 runs compared to measured values available.

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Special Topics Research

The PD/A CRSP also conducts research activities not described in workplans. These activities represent an additional effort from CRSP researchers and students, and are reported as Special Topic Research. During this reporting period the potential use of bull testes for sex-reversal of tilapia is being investigated at Auburn University. In addition, researchers are investigating the influence of the treatment environment (aquaria versus hapas) on the efficacy of 17α-methyltestosterone, a commonly used substance for sex-reversal.

Sex Reversal of Tilapia: 17-α Methyltestosterone Dose Rate by Environment, and Efficacy of Bull Testes

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Introduction

Sex reversal of newly hatched tilapia generally is accomplished via oral administration of 17-α methyltestosterone (MT), which has been incorporated into a starter fish feed at 60 mg MT/kg feed (Popma and Green, 1990). While use of the 60 mg MT/kg feed dose consistently yields populations comprised of less than 5% females (i.e., > 95% males), this has not been shown to be the optimal dose. Other investigators have reported sex reversal of tilapia at dose rates less than 60 mg MT/kg feed (Guerrero, 1975; Tayamen and Shelton, 1978; McGeachin et al., 1987; Jo et al., 1988; Varadaraj and Pandian, 1989). However, results from some of these studies are inconsistent, and it is difficult to separate treatment environment effects. Thus, it is necessary to identify the optimal dose of MT for consistent, successful sex reversal in a variety of treatment environments.

Naturally occurring sources of testosterone may be an alternative to using a synthetic androgen, which also is an anabolic steroid, for tilapia sex reversal. Haylor and Pascual (1991) reported successful tilapia sex reversal using ram's testes as a source of dietary testosterone. Bull testes are a by-product of beef industry in the U.S., and are a potential source of dietary testosterone for tilapia sex reversal.

The objectives of this research were to determine the efficacy of sex reversal of different dosage rates of MT to fish treated in different environments, and to evaluate the potential of freeze-dried bull testes as a dietary source of testosterone for tilapia sex reversal.

Materials and Methods

Newly hatched Nile tilapia (*Oreochromis niloticus*) were stocked at 8 fry/L into 80-L glass aquaria located inside a hatchery building or into hapas (45-L volume) suspended in 20-m^3 outdoor concrete tanks located at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, AL. Fry were stocked on 1 Aug 1995, and harvested after a 28-d treatment period. Subsamples of fry from each treatment unit were transferred to hapas suspended in 20-m^3 outdoor concrete tanks for nursery rearing to approximately 5-g size. Once fingerlings attained an average weight of 5 grams they were sacrificed, and the gonads excised and gonadal sex determined according to the aceto-carmine squash method (Guerrero and Shelton, 1974).

Trout chow (42% protein) was the carrier for MT, which was incorporated into the feed at 0, 15, 30, 45 or 60 mg MT/kg of feed. The appropriate quantity of MT was dissolved in 500 mL of 95% ethanol/kg feed, and this solution was mixed with the powdered feed. Ethanol only was mixed with feed for the 0 mg MT/kg feed treatment. Ethanol was evaporated from the alcohol-feed mixture, and the dried feed was stored in refrigeration until used. Fry in each treatment were fed at 20% body weight during week 1; the daily ration was divided into four meals. Feed rate was decreased by 2.5%/wk during weeks 2-4. Feed rate was adjusted weekly based on results of weekly population samples.

Frozen bull testes were obtained from a meat packing plant in Montgomery, AL. Individual testes were skinned, sliced, freeze-dried and ground, and mixed with trout chow either in a 1:1 or 1:3 freeze-dried testes:trout chow ratio. Mixed feed was refrigerated until fed and feed was offered as described above.

Results and Discussion

The sex reversal period was completed and the nursery rearing phase initiated by the end of August 1995. Nursery rearing and gonadal sex determination are expected to be completed by December 1995. Data analyses will be performed upon completion of all data collection.

After the 28-d MT treatment period, fry total length ranged from 32.8-39.6 mm and 40.7-44.3 mm for fry treated in aquaria (indoors) and hapas (outdoors), respectively. Average respective final weight ranges were 0.7-1.0 and 1.2-1.9 g/fry. Fry survival in both environments was low and ranged from 16.7-27.7% and 25.7-43.6% in aquaria (indoors) and hapas (outdoors), respectively.

Fry fed feed containing bull testes were 55.6 and 59.7 mm total length for 1:1 and 1:3 ratio feeds, respectively, following the 28-d treatment period. Mean final weights were 2.0 and 0.7 g/fry for 1:1 and 1:3 ratio feeds, respectively, which undoubtedly reflected the difference in respective survival during treatment (28.3% versus 69.2%).

Anticipated Benefits

Results of these trials should indicate the effects of treatment environment on efficacy of tilapia sex reversal using MT. In addition, information on dose rate response should help narrow the range of dose rates that need to be examined to determine the optimal dose rate. Information on the efficacy of using freeze-dried bull testes as a dietary ingredient for sex reversal of tilapia may offer an alternative to using dietary MT.

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Appendix A: Acronyms

A

AIT	Asian Institute of Technology, Thailand
AID	now USAID
ALCOM	Aquaculture for Local Community Development
ANDAH	National Association of Honduran Aquaculturists
ANOVA	analysis of variance
APHA	American Public Health Association

B

BOD	biological oxygen demand
BW	body weight

C

C	carbon
$CaCO_3$	calcium carbonate
CCHL	carbon:chlorophyll *a* ratio
CEC	cation exchange capacity
CLSU	Central Luzon State University
CM	chicken manure
CO_2	cabon dioxide
COD	chemical oxygen demand
CRSP	Collaborative Research Support Program

D

DAST	Data Analysis and Synthesis Team
DIC	dissolved inorganic carbon
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
DO	dissolved oxygen
DOF	Royal Thai Department of Fisheries, Thailand
DOS	operating system of IBM and IBM compatible computers
DPF	days post fertilization
DSS	decision support system

F

FAC	Freshwater Aquaculture Center, Central Luzon State University, Philippines
FAO	Food and Agriculture Organization of the United Nations
FCR	feed conversion rate

G

GA	genetic algorithm
GCE	gross conversion efficiency
GIFT	genetic improvement of farmed tilapia
GIS	geographic information system
GIT	genetic improvement of tilapia
GMT	genetically produced male tilapia
GPP	gross primary productivity

H

HC	host country
HC PI	principal investigator in a host country
HOD	hypolimnetic oxygen deficit
HPLC	high performance liquid chromatography

I

ICLARM	International Center for Living Aquatic Resources Management
INAD	Investigational New Animal Drug permit

L

LDC	lesser developed countries
LR	lime requirement

M

MB mibolerone
MT 17α-methyltestosterone
MDHT 17α-methyldihydro-testosterone

N

N nitrogen
NDF non-digested feed
NGO Non-Governmental Organization

O

OIRD Office of International Research and Development
OSU Oregon State University

P

P phosphorus
PD/A CRSP Pond Dynamics/Aquaculture Collaborative Research Support Program
PI Principal Investigator
PMO Program Management Office
POND decision support software, developed by the PD/A CRSP
PONDCLASS expert system software, developed by the PD/A CRSP

R

RA Research Assistant

T

TAN total ammonia nitrogen
THB Thai baht, currency of Thailand
TMP triple monophosphate
TSP triple superphosphate
TSS total suspended solids

U

UCD University of California at Davis
USA United States of America
USAID United States Agency for International Development
USDA United States Department of Agriculture
USPI United States Principal Investigator

W

WCR water column respiration
WID Women In Development, USAID

WWW World Wide Web

Appendix B: Table of Contents of the 13th Administrative Report

I. Introduction 1

Historical Overview 1
New Activities 3
Continuing Activities 4
CRSP Continuation Plan 6
CRSP Administrative Reports 7

II. Summary of Activities and Accomplishments 8

Global Studies & Activities 8
Central America 10
East Africa 12
Southeast Asia 13
United States –
Data Analysis and Synthesis 16
Special Topics Research 17

III. Program Background 18

The CRSP Research Program 18
CRSP Work Plans 19

IV. Abstracts of Technical Papers 20

A. Global Experiment and Related Investigations 20
Effect of Pond Management Strategy on Nutrient Budgets 20
Experimental Evaluation of Lime Requirement Estimators for Global Sites 20
Experimental Evaluation of Lime Requirement Estimators for Global Sites- Isolation Column Experiment 21
Phosphorus Adsorption Capacity and Availability of Added Phosphorus in Soils from Aquaculture Areas in Thailand 21
Problem Perceptions, Production Practices, and Economic Incentives for Tilapia Producers in Four PD/A CRSP Countries 22

Central Data Base 23

B. Central America 23

Characterization of Shrimp Farm Effluents in Honduras and Chemical Budget of Selected Nutrients 23
Relationships among Stocking Density, Survival and Yield in Ponds Affected by the Taura Syndrom During Wet and Dry Seasons in Honduras 24
Nitrogen Fertilization in the Presence of Adequate Phosphorus 24
Effect of Diet Protein on Food Conversion and Nitrogen Discharge during Semi-Intensive Production of *Penaeus vannamei* 25

C. East Africa 26

Masculinization of Nile Tilapia (*Oreochromis niloticus*) through Immersion in 17α-Methyltestosterone or 17α-Methyldihydrotestosterone 26
Maximum Voluntary Feed Intake and Growth of Nile Tilapia Fry as a Function of Water Temperature 26
Characterization of African Soils and Site Evaluation 27
Reproductive Efficiency of Nile Tilapia (*Oreochromis niloticus*) and Red Tilapia (*Oreochromis spp.*) and a Comparison of Fry Growth and Susceptibility to Being Sex Reversed 28
Growth and Efficiency of Sex Reversal of Nile Tilapia (*Oreochromis niloticus*) Fed 17α-Methyltestosterone-Treated Feed Stored Under Different Storage Regimes 28

D. Southeast Asia 29

The Effects of Fertilization on Growth and Production of Nile Tilapia in Rain-Fed Ponds 29
A Finishing System for Large Tilapia 30
Stocking Density and Supplemental Feeding 30
Carp/Tilapia Polyculture on Acid-Sulfate Soils 31
Diel Cycles of Temperature and Dissolved Oxygen Stratification in Deep Rain-fed Ponds 31
Carbon Dioxide Exchange between Pond Water and the Atmosphere 32
Growth Comparison of Three Strains of Nile Tilapia in Fertilized Ponds 32
Polyculture in Deep Ponds 33
Outreach Assistance 33
Effect of Pond Size 34

E. United States 34

Decision Support for Pond Aquaculture:
Simulation Models and Applications 34
Decision Support for Pond Aquaculture:
Parameter Estimation Techniques 35
Aquaculture Pond Modeling for the Analysis
of Integrated Aquaculture/Agriculture Systems 35

F. Special Topics Research 36

Stochastic Modeling of Temperature and
Dissolved Oxygen in Stratified Fish Ponds 36
Sex Reversal of Tilapia: 17-α Methyltestosterone Dose
Rate by Environment, and Efficacy of Bull Testes 36

V. Public Service 38
Institution Building 38
Education and Professional Development 39
Thailand, Honduras, and United States 39
Degree programs 40
Linkages 42

VI. Project Development 45
Development of Sustainable Aquaculture Systems 46
Socioeconomic Studies 47
Participation in International Scientific Meetings and Conferences 48

VII. Program Management and Technical Guidance 51
Advisory Groups 54
Board of Directors 54
Technical Committee 56
External Evaluation Panel 58
CRSP Administrative and Technical Reports 60

VIII. Financial Summary 64

IX. Staff Summary 66

X. CRSP Publications 70
Data Analysis and Synthesis Team 70
Egypt 72
Honduras 73
Indonesia 76
Panama ~ Aguadulce 78
Panama ~ Gualaca 79
Philippines 80
Rwanda 81
Thailand 84
Research Reports 88
Data Reports 90
Other Publications 91

Appendices

A. Acronyms 93
B. Contents of Thirteenth Annual Technical Report 95

Appendix C: Egypt Final Report

The Egypt Project, a collaborative research endeavor, initiated in October 1992 and completed in March 1995, culminated in the development of a cohesive, multi-faceted research program. The program activities included: (1) the testing of guidelines developed for tropical aquaculture, (2) bioconversion and polyculture research, and (3) biotechnology research. The final report for the Egypt Project is appended to this document in its originally published form.

EGYPT PROJECT FINAL REPORT

CONTENTS

EXECUTIVE SUMMARY 1

PROJECT HIGHLIGHTS 2

LIVING OFF THE RIVER 3

RAISING FOOD FISH 3

TAPPING OPPORTUNITIES 4

ADVANCING EGYPTIAN AQUACULTURE 4

COMBINING EXPERIENCES 5

OPTIMIZING THE POND ENVIRONMENT 7

INNOVATIONS IN BIOTECHNOLOGY 8

RESEARCH SUMMARIES 9

ADDITIONAL ACHIEVEMENTS 22

BOLSTERING INSTITUTIONS 22

AMPLIFYING RESULTS 22

ENDURING CONTRIBUTIONS 24

INSTITUTIONAL COLLABORATION 25

PROJECT PARTICIPANTS 26

FINANCIAL SUMMARY 30

The fish are growing "Better than wonderful"

(comment by Egyptian CRSP Participant)

Hillary Egna (CRSP Director), Bartholomew Green (CRSP Egypt Project Chief-of-Party), and Abbassa station researchers Fatma Hafez, Abdel Mostafa, Hussein El Ghobashy, Ali Abdelghany, Zeinab Elnagdy, Hussein Hebicha, Gamal El Naggar (from left to right) gather in front of the newly renovated ponds at the Central Laboratory for Aquaculture.

EXECUTIVE SUMMARY

The Egyptian Central Laboratory for Aquacultural Research (CLAR) and the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP), during two and one-half years of collaborative research, combined experience and expertise to develop a cohesive, multi-faceted research program. Activities conducted under the program included: (1) the testing of guidelines developed for tropical aquaculture, (2) bioconversion and polyculture research, and (3) biotechnology research. Most of the research was conducted at CLAR, a state-of-the-art research facility located at Abbassa.

For 12 years, CRSP scientists have been conducting research aimed at improving the management of pond systems through the study of pond dynamics in diverse geographic regions. They developed a set of guidelines and models for efficiently programming pond inputs to optimize aquacultural operations. During the Egypt project, researchers compared the effectiveness of CRSP-developed management guidelines with standard Egyptian fish farming practices. This research resulted in the identification of management strategies that save resources through input reduction and improved production. Results from these experiments are entered into the CRSP Central Data Base, the world's largest standardized database on tropical aquaculture, which is accessible to aquaculturists around the world.

Bioconversion studies focused on increasing pond productivity through the enhanced use of underutilized resources. A first set of experiments showed that grass and black carp feed on aquatic vegetation and snails found in Egyptian ponds. A second series of investigations evaluated the suitability of grass and black carp as new components in an Egyptian polyculture system composed of native species such as tilapia, mullet, and catfish. Results indicated that using catfish as a predator for unwanted tilapia reproduction in a polyculture system proved to be less productive than stocking ponds with all-male tilapia.

The Egypt project's biotechnology experiments focused on the practical application of modern technology to biological systems. Researchers identified a binding site for the synthetic androgen mibolerone and studied using short-term immersions of tilapia fry in 17α-methyltestosterone (MT) and 17α-ethynylestradiol to evaluate the efficacy of

these treatments for masculinization and feminization. Identification of the mibolerone binding site is a first step toward understanding how steroids cause sex inversion in fish, and may provide a possible tool for screening potential sex-inverting compounds. In a series of hatchery studies the growth-promoting effects of MT were differentiated from its sex-reversing effects. Lastly, scientists tested the progeny of tilapia breeding groups to identify YY male tilapia among broodfish. This effort is a step towards developing a YY tilapia breeding program, and may make a contribution to our ability to produce male tilapia for public consumption that have not been treated with androgenic hormones.

While the primary focus of the Egypt project was collaborative research, institutional and professional development, and the establishment of new linkages were also of importance. It was also envisioned that the Egypt project would act as a catalyst to propel the Abbassa facility to a prominent role in aquaculture research and training, and has been successful in these areas, too. Currently, CLAR is being considered as a new site for the International Center for Living Aquatic Resources Management (ICLARM). The CRSP Egypt project supported through its Scholarly Exchange Program the professional development of Egyptian scientists by sponsoring 21 visits to laboratories and 12 visits to international conferences. Fourteen students, including eight working on advanced degrees, were supported by the Egypt Project. Over 160 individuals participated in CRSP sponsored workshops at CLAR. The CRSP Egypt project also assisted with facility improvement and maintenance, e.g. the renovation of experimental ponds and an overwintering facility.

PROJECT HIGHLIGHTS

— Improved Egyptian aquaculture techniques by providing and applying valuable information about pond dynamics.

— Determined economic returns of different management strategies for Egyptian pond production.

— Transferred CRSP technology for efficient tilapia production to Egypt.

— Described production characteristics of two tilapia species native to Egypt.

— Collected baseline information on Egyptian pond soils.

— Demonstrated the usefulness of grass carp as a biological control agent for vegetative regrowth.

— Collected baseline information on feeding behavior of black carp.

— Advanced efforts towards the creation of an Egyptian polyculture system.

— Advanced state-of-the-art biotechnological aquaculture research.

— Aided in the ongoing effort to obtain U.S. Food and Drug Administration approval of 17 α-methyltestosterone for sex-reversal of fish by conducting clinical field trials.

— Trained and educated over 160 individuals on a wide variety of topics through the presentation of numerous workshops.

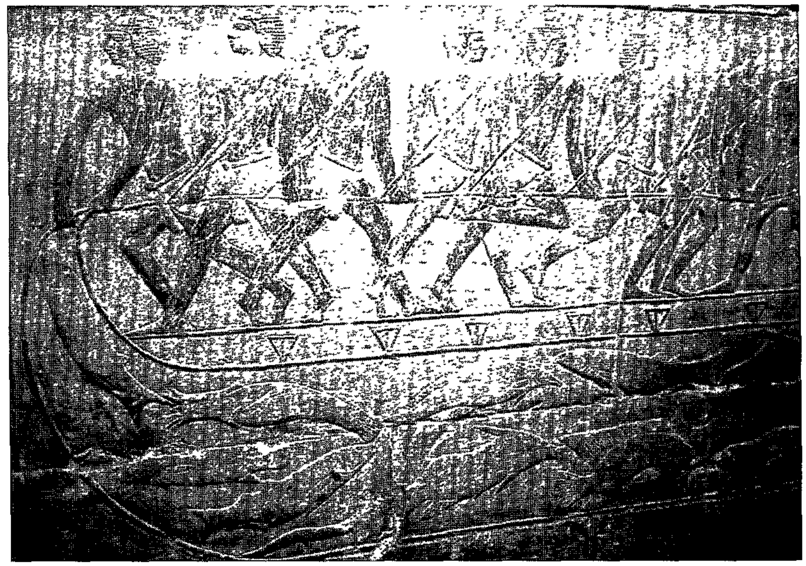
— Facilitated the creation of new networks among aquaculture researchers, thereby contributing to a greater flow of information and increased research efficiency.

— Enhanced the infrastructure at the Central Laboratory for Aquaculture Research in Egypt.

— Increased the visibility of Egyptian aquaculture and aquaculture research.

LIVING OFF THE RIVER

Egypt, located in the northeast corner of Africa, covers over one million square kilometers, yet only three percent of Egypt's total land area is classified as agricultural land. Most of that land is distributed in the Nile Valley, which constitutes only four percent of Egypt's land area and where over ninety eight percent of the population is located. The Nile River, the longest river in Africa, flows through Egypt for 1,600 life-giving kilometers. Fifty one percent of the labor force is employed in agriculture. However, per capita availability of domestically produced food has been decreasing, and imported food products currently represent one-third of Egypt's total imports, one of the highest food import ratios in the world.



Bas-relief from Thebes showing a fishing scene from 4000 years ago. Tilapia, one of the fish pictured, have been valued by Egyptians since these ancient times.

Fish is an important component of the Egyptian diet. It currently accounts for about thirty percent of total animal protein consumption in Egypt. To meet this demand, Egyptians depend on capture fisheries and aquaculture. However, because capture fisheries have declined and aquaculture production has not kept pace with demand, Egyptians increasingly rely on imported fish. Of the 350,000 metric tons of fish consumed in 1989, over 100,000 metric tons were imported, and only 33,000 metric tons were produced through domestic aquaculture.

RAISING FOOD FISH

Aquaculture has been practiced in Egypt for thousands of years. Historically, Egyptian aquaculture consisted of the howash method of fish farming. A howash is a low lying enclosure which fish enter when the land is flooded. After the water level has dropped, farmers harvest the trapped fish. This method of fish-farming has been replaced in this century by more intensive management systems. Currently, Egyptian pond aquaculture involves a polyculture of mixed-sex, young-of-the-year tilapia, mullet, and common and silver carps. Natural food production is stimulated by fertilization, but fish are also fed a commercial food ration.

Over the last decade the supply of fish from aquaculture has increased. However, this rise in supply has resulted primarily from increasing the area of production, rather than from improving yield. Opportunities to further increase the area of production are limited for several reasons. Even though Egypt is land-rich, land in close vicinity to the river or irrigation canals—the prime locations for fish farms—is also in high demand for agriculture and housing. Further, water is a precious resource in arid Egypt and aquaculturists face many competitors for this resource. Therefore, the route to increased fish production will require that pond productivity be improved.

Recognizing the need for improved aquaculture technology, the Egyptian government, with financial support from the U.S., established the Central Laboratory for Aquaculture Research at Abbassa (CLAR). CLAR, with its state-of-the-art facilities and equipment, attracted the attention of international research programs and entered into a partnership with Pond Dynamics/ Aquaculture Collaborative Research Support Program (PD/A CRSP) scientists to tap into the resources and opportunities provided by this U.S.-based program.

TAPPING OPPORTUNITIES

The CRSP is an international effort to develop aquacultural technology as a means of confronting food and nutritional problems worldwide. The CRSP is funded by the U.S. Agency for International Development (USAID), under the authority of the International Development and Food Assistance Act of 1975 (P.L. 94-161), and by the universities and institutions that participate in the CRSP. Oregon State University is the Management Entity for the CRSP and has technical, administrative, and fiscal responsibility for the performance of grant provisions.

The CRSP is a cohesive program of research carried out in selected host countries and the U.S. by teams of U.S. and host country scientists. The resources of U.S. and host country institutions are brought together to improve the efficiency of pond culture systems through sustainable aquaculture. The strategy adopted by the CRSP has been to undertake the basic research required to improve the efficiency of pond culture systems.

CRSP research projects have focused on improving the management of pond systems through the study of pond dynamics since 1982. CRSP research successes are well documented, and include guidelines for efficiently programming inputs to optimize aquacultural operations. Using these guidelines, yields of five times the amounts previously attained were experienced at some CRSP research sites. CRSP researchers have also demonstrated that low-cost agricultural products and by-products can be used as successful substitutes for pelleted fish feed, often at considerable savings. In another important research project, CRSP scientists extensively researched water quality issues, resulting in improved pond management and reduced environmental impacts. Additionally, CRSP scientists have developed hatchery techniques that enable countries to provide enough seed fish to "jump-start" their aquaculture industries. Results of these CRSP experiments have yielded valuable information for private aquaculture—information that has translated into significant economic returns.

ADVANCING EGYPTIAN AQUACULTURE

When the CRSP joined the Egyptian effort to increase the availability of animal protein through pond aquaculture in Egypt, an ambitious and exciting endeavor began. Egyptian and CRSP scientists developed a comprehensive plan which fully utilized the facilities provided by CLAR as well as the CRSP's twelve years of experience in aquaculture research by carrying out innovative research of immediate scientific interest and practical application. The expected outcomes included advancements in current understanding of pond dynamics, improvement of aquaculture techniques and production, and greater visibility for Egyptian aquaculture. The evidence of success is abundant.

The plan to further aquaculture production and technology in Egypt consisted of a coordinated effort on three related fronts. First, the CRSP Egypt project investigated increasing pond production by adapting the CRSP's extensive research results from tropical countries to Egypt. Second, studies in bioconversion attempted to optimize pond production by utilizing available energy sources, such as snails and pond vegetation, while at the same time reducing pond management problems caused by these organisms. In a related vein, polyculture studies focused on increasing fish yields by combining the species used in the bioconversion studies with traditional aquaculture species. Finally, biotechnology studies investigated various techniques for producing all-male tilapia in order to limit unwanted reproduction in ponds which reduces fish quality.

In addition, the CRSP Egypt project strengthened institutional capacities, promoted education and professional development, established international scientific linkages, and furthered the exchange of technical information. These ancillary benefits increased the impact and scope of the CRSP Egypt project.

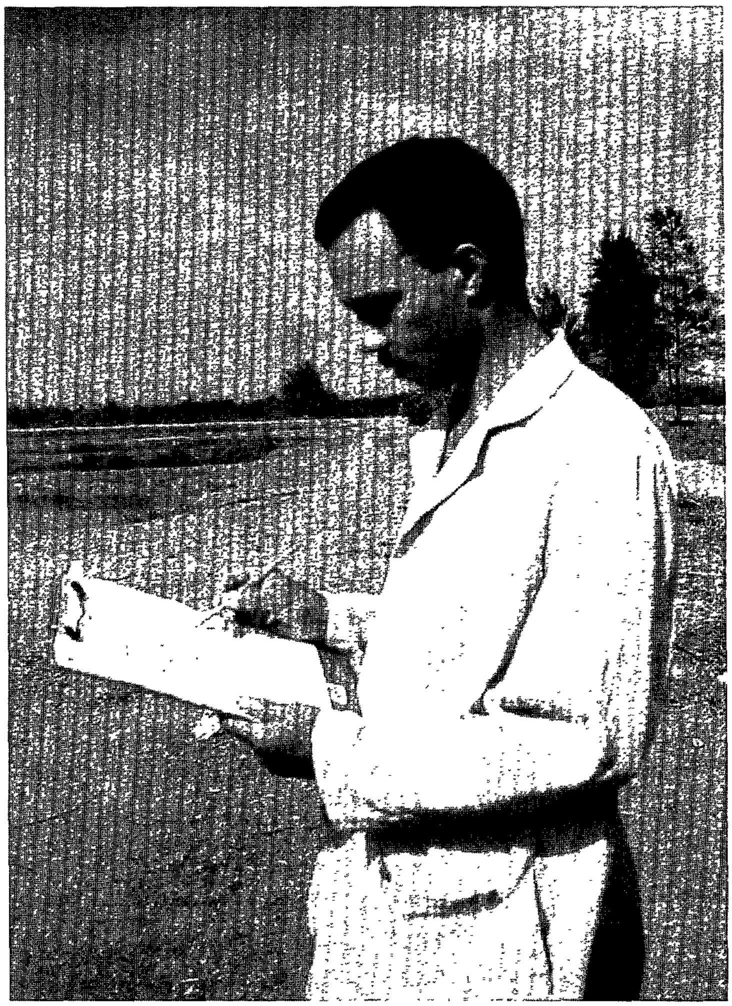
COMBINING EXPERIENCES

The ancient practice of Egyptian fish farming has created a wealth of information. Yet, this information was often anecdotal, incomplete, and limited to extensively managed systems. Since the beginning of this century, more intensive management practices have become commonplace in agriculture enterprises. Egyptian fish farmers, like their international colleagues, increased fish yields by adopting more intensive management practices such as fertilization and feeding. However, Egyptian aquaculturists had not attempted to systematically compare Egyptian management practices to aquaculture techniques used in other countries.

The CRSP Global Experiment uses Nile tilapia (*Oreochromis niloticus*) as the test species. Although the Nile tilapia is one of Egypt's native fish species, the Nile delta region is the most northern border of its natural range. Occasionally, unseasonably cold winters can kill unprotected stocks of Nile tilapia in the delta region, thus jeopardizing local aquaculture operations that rely on this species. Another indigenous species, the blue tilapia (*O. aureus*), is considerably more cold tolerant. However, knowledge of its production characteristics was limited. Therefore, it was not known if blue tilapia could be substituted for Nile tilapia in somewhat harsher environments without experiencing yield reductions. A thorough comparison of Nile tilapia and blue tilapia performance under the same production regime was therefore necessary.

Research Efforts

Two experiments were conducted that specifically fit within the parameters of the CRSP Global Experiment. Each experiment was designed to shed light on different aspects of pond management strategies in Egypt. The first experiment focused on validating CRSP nutrient input strategies and evaluating their economic efficiency. The second Global Experiment focused on comparing production characteristics and economics of two different species of tilapia under different pond conditions. Two other studies, in addition to those in the workplan, constituted further effort on the part of the Egypt project scientists. The first experiment compared the effects of stocking rates on growth and yield of Nile tilapia; and the second collected baseline information on chemical and physical characteristics of pond soils in Egypt.



Dia Kenawy records fertilizer applictions to experimental ponds during the Global Experiment.

183

Through routine water quality analysis researchers assure that pond conditions do not deteriorate beyond specified limits.

Global Experiment Research Impacts

— The addition of a commercial ration is not necessary during the first two months of fish culture because natural productivity, resulting from fertilization, is sufficient to maintain fast fish growth during the first two months of culture.

— Fertilizer application beyond day 60 in the "Traditional Egyptian" treatment is not necessary.

— Net tilapia yield from ponds fertilized with a high rate of chemical fertilizer is similar in Egypt, Honduras, and Rwanda, and less than in Thailand.

— Young fish probably are unable to consume commercial feed pellets available in Egypt because of the relatively large particle size of the pellets.

— Nile and blue tilapia grown under the same management system showed no significant yield differences. However, Nile tilapia yielded a significantly higher percentage of larger fish than the blue tilapia.

— Pond production strategies based on past CRSP research produce a greater percentage of large tilapia than traditional Egyptian treatments.

— Pond production strategies based on past CRSP research result in significantly less unwanted reproduction during grow-out than other pond production techniques.

— Net returns to land and management are highest with a CRSP pond production strategy.

— A positive relationship exists between the intensity of the pond production system and the average price received for fish.

— Although a less intensive pond management strategy (chemical fertilization using monosex tilapia) has the lowest variable cost, more intensively managed systems following CRSP guidelines have a higher average return per kilogram of fish.

— Semi-intensive pond production strategies can tolerate a greater reduction in average price before net returns become negative.

— The greatest total production, net returns and average rates of return on capital were obtained in semi-intensive production systems. Semi-intensive production strategies produced the highest values of production per labor-hour, per kilogram of feed, or per Egyptian-pound of variable cost. They also had the highest margin between average price and break-even price.

— Pond soils in Egypt had greater concentrations of sulfur, calcium, magnesium, potassium, and sodium, and lower concentrations of iron, manganese, zinc, and copper than pond soils from a humid climate.

— For general purposes, soil sampling should be restricted to the upper 5 cm layer.

OPTIMIZING THE POND ENVIRONMENT

Aquatic plants and snails are abundant in many fish ponds in Egypt and represent a nutrient and energy sink that tilapia cannot use for growth. In addition, several species of snails present a human health hazard because they are hosts to the parasite that causes bilharzia. The aim of CRSP bioconversion research was to select and study fish species that could convert the immobilized nutrients and energy in plants, snails, and unwanted tilapia fry into fish flesh. At the same time, pond management problems associated with plants, snails, and tilapia young were expected to be reduced.

Grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) were the species identified to bioconvert the plant and snail biomass, respectively. The African catfish, *Clarias*, was selected as a predator of tilapia young. Grass carp were selected because of their unique ability to process large amounts of aquatic plants. Furthermore, by their mechanical processing and digestion, grass carp release and recycle nutrients retained in the plants. The released nutrients stimulate production of preferred plankton communities. Although even grass carp cannot directly utilize the mature high fiber nuisance plants prevalent in Egyptian ponds, CRSP research focused on consumption of new growth after mechanical cutting had taken place. Black carp were selected because they are voracious snail predators. Several snail species are intermediate hosts for the parasite that causes bilharzia. Black carp predation on snails was thought to result in a reduced risk of infection.

The information gathered from the bioconversion studies was to provide the foundation for the development of a polyculture system. The objectives of the polyculture studies were to test an array of species combinations and stocking rates that would optimally utilize the available energy and nutrient supplies under pond conditions in Egypt.

Polyculture is another mechanism for fully using a three-dimensional growing space by exploiting the entire water column. Although other forms of agriculture have used three-dimensional growing spaces aquaculture is alone in the widespread commercial application of this technique. Egyptian aquaculture has traditionally used multispecies stocking; however, simply mixing several species within a single pond does not produce a polyculture system. In order to form a polyculture system, each species must have a function that does not negatively interfere with other constituent species and, more importantly, each species must contribute to mutualistic effects, where positive interactions increase production.

Research Efforts

Initial bioconversion experiments focused separately on (1) grass carp growth from plants, (2) black carp growth from snails, and (3) the effect of stocking density on the biomass of plants and snails. Experiments evolved to study pond production when both species were stocked, with and without supplemental feeding. It was necessary to consider the potential synergism between black and grass carp because plants provide food and cover to snails and thus impact the system. The final bioconversion study focused on the African catfish, *Clarias*, as a bioconverter of unwanted tilapia offspring.

The objectives of the polyculture studies were to test various combinations of grass carp, black carp, tilapia, *Clarias*, and mullet. The studies were designed to determine whether any of these species combinations had greater yields due to synergistic effects. In addition, researchers conducted a study on black carp feeding biology which was not included in the original workplan. Polyculture experiments had to be terminated prior to the proposed evaluation date of the experiments, thus limiting the usefulness of collected data.

Bioconversion and Polyculture Research Impacts

— Grass carp effectively maintain ponds free of emergent vegetative regrowth.

— Grass carp stocking needs to occur immediately after pond filling.

— Black carp are voracious snail predators.

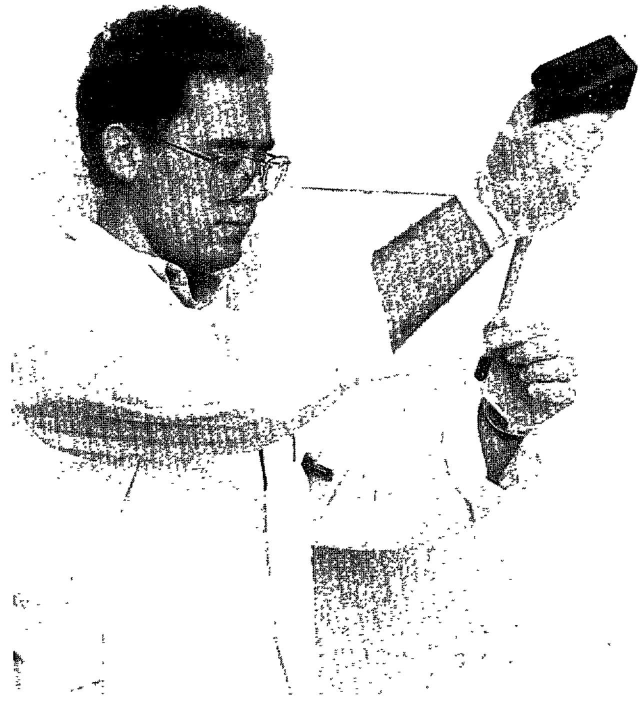
— Black carp growth is reduced in ponds that contained common carp and/or mullet.

— Total length of prey and mouth gape of black carp are directly and linearly correlated.

— Black carp start feeding on mollusks only after they have grown to 160-170 mm in length.

— *Clarias* control tilapia fry populations effectively. However, predation occurs only in semi-intensive culture systems that receive fertilizer but no feed.

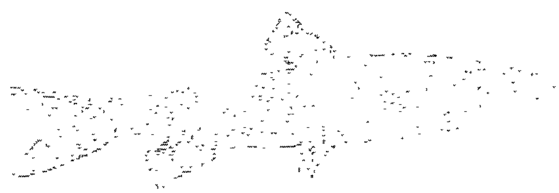
— Tilapia production in polyculture with *Clarias* is significantly lower than in either all-male or mixed-sex culture systems.



As a result of his research on the Egypt CRSP Project, graduate student William Gale received the 1995 Hugo Krueger Fish Physiology Award from Oregon State University.

INNOVATIONS IN BIOTECHNOLOGY

The practical application of modern technologies to aquaculture is essential for improving pond production efficiency. A recurrent problem of tilapia culture is related to reproductive behavior. Tilapia attain sexual maturity at an early age and have the ability to spawn repeatedly during a culture cycle. This leads to pond overcrowding, which results in the production of many small fish of little market value. Therefore, methods for controlling reproduction are essential for tilapia systems. Stocking of all-male fish has proved successful in mitigating the negative impact of reproduction on yield of marketable fish. All-male fish can be produced economically using sex-inverting substances. However, this is still a relatively new technology and many questions remain as to the safest and most effective method of producing mono-sex tilapia.



Research Efforts

CRSP biotechnology studies, while diverse, were centered on refining current means of producing and identifying male tilapia. In particular, CRSP studies focused on the use of sex-inverting substances to produce all-male stocks. Research separated the growth-promoting from the sex-reversing effects of 17α-methyltestosterone (MT), determined the androgen-binding characteristics of tilapia gonadal tissue, tested immersion in an androgen-containing solution as an alternative method of producing mono-sex fingerlings, and worked towards the production of YY supermales.

The CRSP also participated in clinical field trials to investigate the safety of using MT for sex reversal of newly hatched tilapia. These experiments supported efforts of Auburn University, the American Tilapia Association, and a commercial feed producer to collect data to obtain approval from the U.S. Food and Drug Administration (FDA) to use this drug. This research was carried out under a "compassionate" Investigational New Animal Drug exemption granted by the FDA. The field trial required the production of large amounts of fry for sex-reversal. An additional study, not part of the original research plan, was conducted to determine the relationship between fry production and water temperature.

Biotechnology Research Impacts

— A receptor assay developed for tilapia may be used as a tool for screening newly developed sex-inverting agents.

— Immersion in an androgen-containing solution resulted in masculinization of tilapia fry.

— MT significantly increases the growth rate of *O. aureus* and *O. mossambicus* under hatchery conditions.

— In freshwater, *O. aureus* grows nearly twice as fast as *O. mossambicus* after having been treated with MT.

— Use of MT-treated feed effectively sex-inverted tilapia fry under field conditions.

— Variability in the sex ratio of repeated spawns produced by YY supermales indicates that sex determination is not solely controlled by the male genome.

— Mass production of Nile tilapia and blue tilapia fry for sex reversal is not possible below 100 or above 190 degree-days.

VALIDATION OF CRSP POND MANAGEMENT STRATEGIES

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Abstract

The objectives of this research were to quantify tilapia yields resulting from established Pond Dynamics/Aquaculture Collaborative Research Support Program pond nutrient input strategies under the climatic, edaphic and water quality conditions found in Egypt, and to compare these results to those obtained using traditional Egyptian management practices. Five treatments, each replicated four times, were tested in 0.1-ha earthen ponds. Treatments were: Traditional Egyptian, Enhanced Egyptian, Feed Only, Fertilization then Feed, and Chemical Fertilization. Ponds were stocked with 20,000 *Oreochromis niloticus*/ha; mixed-sex fish were stocked in Egyptian treatments and sex-reversed fish in all others. The duration of the experiment was 145 days.

Nile tilapia gross yield differed significantly among treatments and ranged from 1,278 kg/ha (chemical fertilizer treatment) to 2,877 kg/ha (fertilization then feed treatment). Wild tilapia (*O. aureus, Sarotherodon galilae, Tilapia zilli*) invaded all ponds and contributed 81 to 686 kg/ha to total tilapia yield treatment means. Thus, total tilapia yield ranged from 1,407 to 3,537 kg/ha and represented from 78% to 96% of gross fish yield. Gross fish yields ranged from 1,526 to 4,074 kg/ha. Tilapia yields in the Traditional Egyptian and Fertilizer then Feed treatments were significantly greater than in the Chemical Fertilizer treatment. Tilapia are marketed in Egypt by size class as follows: 1st class – 1 to 5 fish/kg; 2nd class – 6 to

12 fish/kg; 3rd class – 13 to 25 fish/kg; and, 4th class – 26 to 40 fish/kg. Farm-gate prices vary from L.E. 7.85/kg for 1st class tilapia to L.E. 1.75/kg for 4th-class tilapia. Yields of 1st- and 2nd-class tilapia were greater when organic fertilization was used in combination with formulated feeds than when chemical fertilization alone or formulated feed alone were used. Results indicate that ponds stocked with young-of-year monosex tilapia and managed according to the tested systems are feasible in Egypt.

ECONOMIC ANALYSIS OF DIFFERENT TILAPIA POND CULTURE SYSTEMS IN EGYPT

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Abstract

Five different tilapia pond management strategies, tested in 0.1-ha earthen ponds at the Central Laboratory for Aquaculture Research, Abbassa, were evaluated for economic potential. Pond management strategies were Traditional Egyptian, Enhanced Egyptian, Feed Only, Fertilization then Feed, and Chemical Fertilization. Ponds were stocked with either mixed-sex or all-male populations of Nile tilapia. Yield and input data from 145-day pond trials were used to develop full-cost budgets for each management system. Net returns, values for production for major inputs, break-even prices and yields, and average rates of return to capital were estimated for each system based on a 2.1-ha production pond.

Net returns ranged from L.E. 19,102 for the Fertilizer then Feed treatment to L.E. 985 for the Chemical Fertilization treatment. Rates of return to capital for these two management strategies were 29.97% and 2.42%, respectively. Net returns to land and management for the Fertilizer then Feed treatment were, on average, 16.1 times the net returns for the Chemical Fertilization treatment. The Fertilizer then Feed management strategy also had the highest margin between average price and break-even price to cover total cost, which indicated reduced risk to the farmer in the event of a decline in market price. Sensitivity analyses indicated that the Fertilizer then Feed management strategy maintained positive net returns if fish yield decreased by two standard errors and price decreased by 20%.

YIELD CHARACTERISTICS OF TWO SPECIES OF TILAPIA UNDER TWO DIFFERENT POND ENVIRONMENTS

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Abstract

CRSP research designs have been based on the use of Nile tilapia (*Oreochromis niloticus*) as the test species because this species was common to all research sites. In Egypt, Nile and blue (*O. aureus*) tilapia, both good culture species, are endemic. Severe cold weather occurs periodically in the Egyptian delta during the winter months of December through February, and can decimate unprotected stocks of Nile tilapia, as happened during the 1991/92 winter. *Oreochromis aureus*, much more tolerant of cold temperatures, survives winters in Egypt without having to be over-wintered indoors. The increased cold

188

tolerance of *O. aureus* would give this species a competitive advantage over *O. niloticus* for pond culture in the Egyptian delta assuming all other production characteristics were similar. The objective of this experiment was to compare production characteristics and production economics of *O. niloticus* and *O. aureus* reared in ponds managed under two different nutrient input regimes.

Eighteen 0.1-ha ponds at the Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt, were used for this completely randomized design in 2 x 2 factorial arrangement, where factors were tilapia species (Nile or blue) and pond environment (chemical fertilization or fertilization then feed); in addition, three ponds per nutrient input regime were co-stocked with equal numbers of Nile and blue tilapia. Weekly applications of nitrogen at 25 kg/ha and phosphorus to maintain 4 N:1 P were made in the Chemical Fertilizer treatment. In the Fertilizer then Feed treatment, chicken litter was applied weekly at 1,000 kg dry matter/ha for the first eight weeks followed by feed (25% protein commercial fish feed) only. Ponds were stocked with sex-reversed tilapia fingerlings on 1 July 94 at a stocking rate of 20,000 fingerlings/ha. Duration of grow-out averaged 158 days.

Nile tilapia gained a mean of 155 g during the grow-out cycle, not significantly different from the 127 g gained by blue tilapia (P = 0.0642). Fish in the Chemical Fertilizer treatment gained significantly less weight (95 g) than those in the Fertilization then Feed treatment (181 g). In the Chemical Fertilizer treatment, weight gains were 95 g and 96 g for Nile and blue tilapia, respectively. Nile tilapia appeared to gain weight faster than blue tilapia in the Fertilization then Feed treatment although an analysis of the species by pond environment interaction was not significant (P = 0.0562). Weight gains in communally-stocked ponds were similar to those observed in separately-stocked ponds. Nile tilapia gained 106 g and 235 g in Chemical Fertilizer and Fertilization then Feed treatments, respectively. Respective gains for blue tilapia averaged 94 g and 182 g.

Tilapia yield did not differ significantly between species (2,590 kg/ha for Nile tilapia and 2,220 kg/ha for blue tilapia). However, tilapia yield was significantly greater in the Fertilization then Feed pond environment (3,060 kg/ha) compared to the Chemical Fertilizer pond environment (1,646 kg/ha) ($P \leq 0.05$). Mean Chemical Fertilizer treatment yields were 1,455 and 1,773 kg/ha for Nile and blue tilapia, respectively. Yields of Nile and blue tilapia in the Fertilization then Feed treatment were 3,725 and 2,627 kg/ha, respectively. Tilapia survival did not differ significantly between species or in different pond environments, nor was the interaction significant.

In terms of size composition of the harvest, significantly more 1st-class Nile tilapia (1,510 kg/ha) were harvested than blue tilapia (35 kg/ha), and the Fertilization then Feed treatment (1,586 kg/ha) yielded significantly more 1st-class tilapia than the Chemical Fertilizer treatment (5 kg/ha) ($P \leq 0.05$). Significantly greater quantities of 2nd-class blue tilapia (1,115 kg/ha) were harvested than Nile tilapia (46 kg/ha), and the Fertilization then Feed treatment (1,344 kg/ha) yielded significantly more 1st-class tilapia than the Chemical Fertilizer treatment (36 kg/ha) ($P \leq 0.05$). There was no significant difference between species for harvest of 3rd-class tilapia (56 kg/ha for Nile tilapia and 73 kg/ha for blue tilapia). However, significantly more 3rd-class tilapia were harvested from the Chemical Fertilizer treatment

Zeinab Elnagdy, a soil scientist and aquaculturist at Abbassa, was one of the Egyptian researchers involved in the Global Experiment.

(1,184 kg/ha) than from the Fertilizer then Feed treatment (14 kg/ha) (P≤0.05).

In summary, there was no significant difference in weight gain between Nile tilapia and blue tilapia, nor was the species by pond environment interaction significant, although in both cases the probability level was very near to the alpha level of 0.05. High variability among ponds, which is commonly observed in pond research, was responsible for lack of significant differences. Given the potential significance of these results, further research is necessary to clarify results of this study, particularly in ponds where supplemental feed is offered to tilapia. Tilapia yields in ponds where supplemental feed is offered were about twice as great as yields obtained in chemically fertilized ponds. Similar yields were observed during Year I (1993) of the Egypt CRSP pond trials.

ECONOMIC ANALYSIS OF TWO TILAPIA POND MANAGEMENT SYSTEMS

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Abstract

The Chemical Fertilizer and Fertilizer then Feed pond management systems tested as part of the 1993 Egypt CRSP Global Experiment were evaluated again as part of the 1994 Global Experiment in order to estimate year-to-year variability. Twelve 0.1-ha earthen ponds at the Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad, were used for this research. Ponds were stocked with all-male populations of Nile (*Oreochromis niloticus*) or blue tilapia (*O. aureus*). Yield and input data from 158-day pond trials were used to develop full-cost budgets for each management system. Net returns, values for production for major inputs, and break-even prices and yields were estimated for each system based on a 1-ha production pond.

Net returns to land management for the Chemical Fertilizer management system were L.E. 168.55/ha for the 158-day production period, while the corresponding value for the Fertilizer then Feed management system was L.E. 10,111.81/ha. Income above variable costs, which is a measure of short-term profitability, was L.E. 2,073.70/ha and L.E. 12,016.97/ha for the Chemical Fertilizer and Fertilizer then Feed management systems, respectively. Returns per kilogram above total variable costs were L.E. 1.26/kg and L.E. 3.92/kg, respectively. Pond management strategy affected the size composition of harvested fish. In chemically fertilized ponds 3% of harvested fish were 1st class, 22% were 2nd class, 72% were 3rd class, and 3% were 4th class. In ponds managed according to the Fertilizer then Feed management strategy 52% of harvested tilapia were 1st class, 44% were 2nd class, and 4% were 3rd class. Average price received for fish sold was L.E. 4.04/kg for the Chemical Fertilizer system and L.E. 6.84/kg for the Fertilizer then Feed management system. The break-even prices to cover total costs were L.E. 3.94/kg and L.E. 3.54/kg for the Chemical Fertilizer and Fertilizer then Feed systems, respectively. The margins between the average price and the break-even price for these respective management systems were L.E. 0.10 and L.E. 3.30.

Comparison of 1993 and 1994 results showed that tilapia yields for each system were similar. However, the size composition of harvested tilapia varied between 1993 and 1994 in both management systems. Third class tilapia were predominant in the harvest from chemically fertilized ponds during both 1993 (51%) and 1994 (72%). Eighty-eight to 92% of tilapia harvested from chemically fertilized ponds were accounted for by the 2nd and 3rd size classes. First-class tilapia comprised 52% of harvested tilapia from Fertilizer then Feed ponds in 1994, up from 24% in 1993; 1st- and 2nd-size classes represented 96% of harvested tilapia in 1994, compared to 69% in 1993. In addition, no 4th-class tilapia were produced in Fertilizer then Feed ponds in 1994.

Two improvements in pond management techniques in effect during the 1994 season, the stocking of known populations of all male tilapia fingerlings and improved exclusion of wild fish from production ponds, contributed

190

to the improved size structure of harvested tilapia. Refinement of feed application rates during the 1994 trial also contributed to reduced feed costs. Fish were fed at 3% of body weight during the 1993 trial, but during the 1994 trial feeding rate began at 3% and decreased progressively to 1% as fish grew. As a result, 40% less feed was used during 1994. In conclusion, the economic viability of the Chemical Fertilizer pond management system appears marginal and quite variable from year to year, while that of the Fertilizer then Feed management system appears profitable with less year-to-year variation.

EFFECT OF STOCKING RATE ON GROWTH AND YIELD OF NILE TILAPIA

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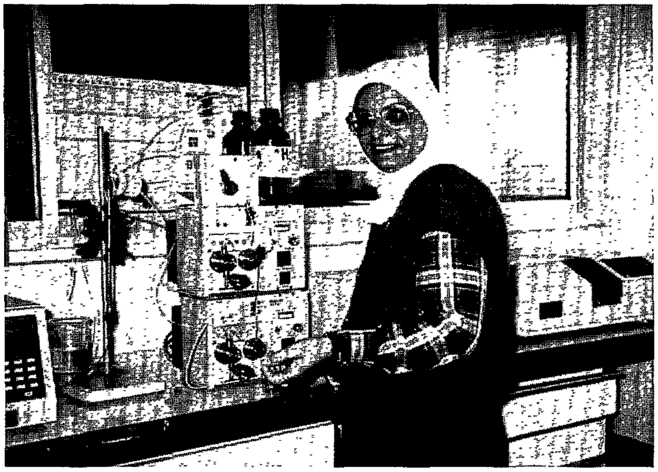
Abstract

Nile tilapia are generally stocked at 10,000-20,000 fish/ha in semi-intensively managed production ponds. Nutrient inputs into these ponds include fertilizers and supplemental feeds; both natural pond productivity and supplemental feed contribute nutrients for fish growth. Often, pond carrying capacity and critical standing crop are not attained during the 5-month grow-out period, which indicates that available pond nutrient resources are underutilized. Knowledge of the pond carrying capacity and density-dependent fish growth for a particular management system would provide the ability to manipulate management to improve production efficiency and economic returns. The objective of this experiment was to quantify growth and yield of Nile tilapia stocked at 30,000 and 40,000 fish/ha in production ponds.

This study was conducted at the Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad, Sharkia, Egypt. Treatments were assigned randomly to three 0.1-ha earthen ponds. Sex-reversed Nile tilapia fry (*Oreochromis niloticus;* mean weight: 0.5 g/fish) were stocked at a rate of 30,000 or 40,000/ha into ponds on 20 and 24 July 1994. Chicken litter was applied weekly at 1,000 kg dry matter/ha for the first eight weeks of the production cycle followed by feed (25% protein commercial fish feed) only. Ponds were harvested 140 days after stocking.

At harvest, it was apparent that high fish mortality had occurred in all ponds. Survival of stocked fish ranged from 9.2% to 58.3%, with mean survival of 37.3% and 15.1% for the 30,000/ha and 40,000/ha stocking rates, respectively. Thus, this experiment provided no data to evaluate the null hypothesis that tilapia growth was not affected by stocking rate. Effective stocking rates were reduced to 11,200/ha and 6,000/ha for the 30,000/ha and 40,000/ha stocking rate treatments, respectively. Tilapia growth at these stocking rates is independent of fish density under the conditions of the present experiment. Individual weight at harvest averaged 176 g and 211 g for the 30,000/ha and 40,000/ha stocking rates, respectively, and did not differ significantly. Possible causes of mortality include stocking mortality, predation on tilapia by the piscivorous African catfish (*Clarias gariepinus*), and poaching. Fry mortality at



Asma Ali El Kerdawy demonstrates the use of state-of-the-art equipment used by researchers in the laboratory at Abbassa.

191

stocking would be difficult to quantify visually because stocked fry averaged 0.5 g each. No mass mortality was thought to have occurred during the grow-out period as dead fish were not observed. A number of African catfish managed to invade the ponds and a mean of 760 kg/ha and 240 kg/ha of African catfish were harvested from the 30,000/ha and 40,000/ha stocking rate treatment ponds, respectively. The quantity of African catfish harvested from ponds did not differ significantly between treatments. However, the individual size of catfish may have affected survival as tilapia survival appeared to decrease as individual catfish size increased from 0.2 to 1.3 kg (P = 0.07). It was impossible to verify whether poaching had occurred.

Joel Watkins (left) of the Salmon River Hatchery in Oregon discusses a fish disease problem with Ahmed Said (center) and Abdel El Gamal. The two Egyptian scientists visited aquaculture facilities in Oregon after they attended the CRSP Annual Meeting in Portland in 1993.

CHEMICAL AND PHYSICAL CHARACTERISTICS OF BOTTOM SOIL PROFILES IN AN ARID CLIMATE AT ABBASSA, EGYPT

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Abstract

Soil cores were taken from ponds at the Central Laboratory for Aquaculture Research, Abbassa, Egypt. Three ponds had received little management since construction in the early 1980s. Three other ponds were fertilized heavily in 1993 and 1994 to stimulate tilapia (*Oreochromis niloticus*) production. A new classification system for aquaculture pond horizons was applied. Thicknesses of S, M, and T horizons in soil profiles averaged 5, 7.5, and 10 cm, respectively. The S horizon contained more silt than clay, but the T horizon and the original pond soil (P horizon) were 60% clay. Concentrations of total carbon, total nitrogen, total sulfur, phosphorus, calcium, and potassium were greatest in the S horizon and lowest in the T horizon. Intensively-managed P-Ponds had higher concentrations of phosphorus and lower concentrations of organic matter and sulfur in S and M horizons than B-Ponds. Compared with pond soils from a humid climate (Alabama, U.S.A.), pond soils at Abbassa had greater concentrations of sulfur, calcium, magnesium, potassium, and sodium, and low concentrations of iron, manganese, zinc, and copper. Over the entire two-year study period at Abbassa, the only changes noted between P-Ponds and B-Ponds was an increase in phosphorus and a decrease in organic matter and sulfur in the heavily fertilized P-Ponds. Because of high moisture content, low dry bulk density, and greater concentrations of organic matter and nutrients in the S horizon, reactions in this layer probably have a greater

192

influence on pond water quality than those in deeper horizons. For general purposes, soil sampling should be restricted to the S horizon or the upper 5 cm layer where depth of the S horizon is not known.

USE OF GRASS CARP AND BLACK CARP IN EGYPTIAN FISH CULTURE

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Abstract

Grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) were evaluated as biological control agents and building blocks for a polyculture system suited to Egyptian conditions. Grass carp were expected to reduce the dependence on mechanical control of plants, concomitantly transforming a management problem and unused resource into an asset. Black carp were evaluated for their potential as control agents of snails, another management problem and unused resource in current Egyptian aquaculture. Several snail species are intermediate hosts for bilharzia, a parasitic disease effecting humans. Interaction between grass carp and black carp was expected to be beneficial as grass carp remove refuges for snails by cropping vegetation. Reduced cover may improve the predacious effectiveness of black carp. These bioconversion experiments were followed by polyculture experiments in which the two species were raised together with additional species (tilapia, mullet, and *Clarias*).

Bioconversion experiments were conducted in two growing seasons, while polyculture experiments were only conducted during the second growing season. Results of the first cycle of bioconversion experiments were inconclusive, due to late stocking resulting from difficulties in obtaining fish and to contamination with "wild species" (mainly common carp). However, stocking proceeded on schedule in the second cycle of experiments and bioconversion ponds were not found to be contaminated with common carp, a suspected competitor for black carp. Polyculture experiments had to be aborted, hence no final data could be collected.

Ponds were prepared by hand-cutting plants (mainly *Typha* and *Phragmites*) to near the soil surface. The ponds were refilled and stocked with grass carp (average size 1 kg) at approximately 275/ha. Black carp (average size 250 g) were stocked at 110/ha. Supplemental feed was given daily at 1% estimated biomass. Three ponds received no feed. The area covered by plants was estimated visually each month. Several snail sampling methods were tried, but none were found to be entirely satisfactory. Final evaluation of snail biomass was achieved by collecting the animals from sample areas of recently dewatered pond bottoms.

Grass carp were effective in maintaining ponds free of emergent vegetative regrowth. A standing crop of about 250 kg/ha grass carp appears to be adequate to maintain ponds clear of nuisance plants, even when supplemental feed is provided. Plant regrowth in the control ponds ranged from 50-95% coverage by the end of the season. Grass carp growth averaged 2.8 g/d in the ponds receiving supplemental feed, but only 0.5 g/d in the non-fed ponds. Black carp survival was low. Growth averaged 2.4 g/d when the fish were fed, but only 1.7 g/d when no supplemental food was provided. Black carp appear to effectively control smaller sized snails. Mollusks were present at substantial levels in several ponds (up to 39/m^2 or 73 g/m^2), however, but only at sizes larger than the black carp could eat. Vector species for bilharzia were found in all ponds and the potential of black carp as a biological control for this disease needs further study.

193

FEEDING BIOLOGY OF BLACK CARP

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Abstract

The black carp (*Mylopharyngodon piceus*), used in the traditional Chinese polyculture system, is a known mollusk predator. It crushes the shells of mollusks with strong, molariform pharyngeal teeth which develop during the first year of life. The pattern of tooth development reflects the dietary change that black carp exhibit during this period. Black carp diet changes from zooplankton to benthic invertebrates and finally to mollusks.

A study was conducted to determine the physical limitation of black carp to engulf mollusks by examining prey size in relationship to the mouth capacity of fish of different sizes. Total prey length (L) was directly and linearly correlated to mouth gape (G) with G=1.60+.0471 L (both measured in mm). The size of a snail that could be eaten was closely related to mouth gape. Black carp ate snails equal to their mouth width and were usually able to crush them. Some would engulf snails larger than their gape, but usually were unable to crush them. Black carp were hesitant to take prey from the water surface, but would eat snail after snail from the bottom to satiation.

Within the fish sizes tested, the approximate range of single-bout satiation was 2-8% of the body weight for fish about 100g body weight (210 mm TL), and upwards of 8-13% in multiple feeding trials which were separated by 3-4 hours. Larger fish appeared to be satiated at slightly lower levels; fish of about 200 g (320 mm) consumed a number of snails which were equivalent to between 1 and 5% body weight. Stomach analysis from pond populations of black carp suggest that fish less than 160-170 mm fed primarily on soft-bodied invertebrates. The gut of larger fish almost always contained mainly the remains of snails.

Martin Fitzpatrick (Oregon State University, foreground) demonstrates how to identify the sex of tilapia fry by gonadal examination during a laboratory class conducted at Abbassa in Fall of 1995.

CLARIAS AND TILAPIA INTERACTION

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Abstract

The precocious breeding behavior of tilapia leading to pond overcrowding is often solved by stocking ponds with tilapia of only one sex. However, production of mono-sex populations requires additional efforts. Another approach to this problem entails co-stocking a predator species together with mixed-sex tilapia. By feeding on tilapia offspring, the predator controls the size of the tilapia population. Using an economically valuable species as biological control also increases financial returns at harvest time. A candidate for this scenario is the African catfish (*Clarias gariepinus*), a species native to Egypt. An experiment was conducted to determine (a) the suitability of *Clarias* for polyculture and (b) the effect of polyculture on tilapia growth. Originally, the experiment was to be conducted at the Central Laboratory for

194

Aquaculture Research at Abbassa, Egypt. However, inclement weather resulted in serious die-offs of Nile tilapia (*Oreochromis niloticus*) and a consequent shortage of tilapia fry. The experiment was therefore conducted at the Asian Institute of Technology in Thailand.

The experiment consisted of three treatments: ponds stocked with male tilapia only, ponds stocked with mixed-sex tilapia, and ponds stocked with mixed-sex tilapia and catfish. Catfish were stocked two weeks after stocking of tilapia to ensure abundant tilapia offspring. Ponds were fertilized with urea and Triple Super Phosphate at 21 and 7 kg/ha/week, respectively. During the first six weeks of the study, the fish were fed with a commercial diet at 1.5% body weight of tilapia/day. Feeding was discontinued after six weeks, as the catfish prefered the feed over the tilapia fry. Standard water quality analyses were conducted biweekly. Comparable water quality values were observed for the different treatments. Growth analysis revealed that fish from ponds stocked only with male tilapia grew better than tilapia from the other two treatments. However, there was no significant difference between mixed-sex tilapia raised with or without catfish. Fry production was significantly greater in ponds stocked with mixed-sex tilapia only compared to the polyculture ponds. Gut analysis of catfish demonstrated that the predators fed on tilapia fry. Mean weight of Nile tilapia and net fish yield were lowest in the polyculture system and highest in the mono-sex culture system.

As part of the Global Experiment researchers regularly monitor fish growth.

USE OF 17α-METHYLTESTOSTERONE FOR TILAPIA SEX REVERSAL

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Abstract

Tilapia aquaculture generally is based on stocking populations of only male fish into production ponds, as males grow significantly faster than females. In addition, stocking only male fish effectively controls the precocious spawning behavior characteristic of tilapia. Populations of all male tilapia may be obtained by one of several methods: manual separation of sexes based on visual examination of external morphology, interspecific hybridization, and sex reversal. At hatch, tilapia are sexually undifferentiated. Sex reversal directs differentiation of gonadal tissue to testes through oral administration of an androgen to newly-hatched tilapia fry. The androgen (17α-methyltestosterone (MT)) is administered orally to newly-hatched fry for a 28-day treatment period and then withdrawn permanently from the diet. A clinical field trial at research institutions and commercial tilapia growers throughout the U.S. and overseas is currently underway to collect efficacy data of MT on tilapia sex reversal under field conditions. Data collected under this clinical field trial will support a New Animal Drug Application to the U.S. Food and Drug Administration for the use of MT for sex reversal of newly-hatched tilapia. U.S. and Egyptian researchers involved with the CRSP participated in the field trial.

195

Eduardo Lopez (Central Luzon State University in the Philippines) and Ali Abdelghany (Central Laboratory for Aquaculture Research, Egypt) discussed tilapia culture during the meeting of the PD/A CRSP Technical Committee in Hilo, Hawaii.

Two trials were conducted to determine the efficacy of MT (60 mg MT/kg feed) in producing populations of Nile tilapia (*Oreochromis niloticus*) or blue tilapia (*O. aureus*) comprised of greater than 95% male fish. Additional objectives were to determine the effect of MT treatment on fry growth, as expressed by final weight and length, and fry survival. The operating null hypothesis was that no differences would be observed between the control and MT treatment groups.

Upon completion of Trial I treatment period, Nile tilapia averaged 0.67 and 0.60 g/fry and 32.0 and 31.3 mm/fry total length for the control and MT treatments, respectively. Differences in mean final weight and length were not significant ($P \geq 0.05$). Mean final individual weight and length for blue tilapia in Trial I were 0.36 and 0.34 g/fry, and 25.9 and 27.5 mm/fry for the control and MT treatments, respectively; no significant differences were detected. Fry survival was not affected adversely by MT treatment for sex reversal; control and MT treatment survival averaged 73.5 and 89.4%, and 96.0 and 91.4% for Nile and blue tilapia, respectively.

In Trial II, mean final weights of Nile tilapia did not differ significantly for control (0.46 g/fry) and MT (0.34 g/fry) treatments. Observed final lengths were 27.9 and 25.1 mm/fry for control and MT treatments, respectively. Fry survival in the control treatment averaged 48.9% and did not differ significantly from the MT treatment average of 63.5% survival. Blue tilapia mean final weights were not significantly different and averaged 0.34 and 0.36 g/fry for control and MT treatments, respectively. Observed final lengths were 27.2 and 28.1 mm/fry, respectively. Treatment differences in survival of blue tilapia fry were not observed; control and MT treatment mean survival were 44.2 and 41.9%, respectively.

Fish in the control treatments ranged from 42% male:58% female to 38% male:62% female; these results are within the expected range for the mean of 50% male:50% female ratio observed for untreated tilapia populations. Efficacy of MT treatment appeared slightly greater for Nile tilapia: male fish comprised 96.0% and 98.9% of treated populations in Trials I and II, respectively. Male fish comprised 84.6% and 65.1% of treated blue tilapia populations in Trials I and II, respectively. Deteriorated water quality in blue tilapia treatment tanks may have affected treatment efficacy, particularly during Trial II.

In conclusion, oral administration of MT to newly hatched tilapia fry for a 28-day period results in fish populations comprised predominantly of male fish. The results of these studies indicate that oral administration of MT does not affect growth or survival of tilapia fry during the treatment period.

MASS PRODUCTION OF NILE TILAPIA (OREOCHROMIS NILOTICUS) AND BLUE TILAPIA (O. AUREUS) FRY IN RELATION TO WATER TEMPERATURE

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Auburn University, Alabama, U.S.A.

Esam H. Rizkalla, Ahmed Nassr Alla, Ahmed Khater, and Abdel R. El Gamal
Central Laboratory for Aquaculture Research
Agricultural Research Center
Ministry of Agriculture and Land Reclamation
Abbassa, Abou Hammad, Sharkia, EGYPT

Abstract

A consistent, reliable supply of fingerlings of the desired species, sex, and size is critical to the success of any

aquacultural enterprise. Of the four tilapia species endemic to Egypt, Nile Tilapia (*Oreochromis niloticus*) and blue tilapia (*O. aureus*) are considered better species for pond culture than *Sarotherodon galilae* and *Tilapia zilli*. Hormonal sex reversal is the most efficient means of mass production of monosex tilapia fingerlings at present. Newly hatched fry 9- to 11-mm total length are fed androgen during a 28-day treatment period. Fry for sex reversal can be mass produced in earthen ponds, but water temperature can affect productivity. The objective of this research is to quantify production of Nile and blue tilapia fry in relation to water temperature in Egypt.

Sixty-three trials were conducted between 28 April 1994 and 24 November 1994 at the Central Laboratory for Aquaculture Research, Abbassa. Nile or blue tilapia broodfish were stocked into small (0.01 ha) earthen ponds at a rate of 1.5 female:1 male. Water temperatures in three ponds were monitored continuously by a computerized data logger. Mean hourly water column temperature was calculated. Degree days were calculated by subtracting the base temperature (15°C) from the mean daily water temperature. Accumulated degree-days for each trial were obtained by summation.

Fry production for all trials totaled 322,814 for Nile tilapia and 282,360 for blue tilapia. A mean of 10,087 Nile tilapia/0.01 ha and 9,108 blue tilapia/0.01 ha were obtained at each harvest. Target fry production, i.e., fry of suitable size for sex reversal, totaled 216,900 Nile tilapia and 209,112 blue tilapia. Mean harvest of target fry was 7,746 Nile tilapia/0.01 ha and 7,468 blue tilapia/0.01 ha. Production of both total and target blue tilapia was more variable than that of Nile tilapia.

No Nile tilapia fry production was observed at fewer than 115 degree-days. At greater than 100 degree-days total Nile fry production (expressed as fry/g female) increased significantly with increased degree-days ($Y = 0.013x - 1.445$, $r^2 = 0.563$, $p \leq 0.01$). Above 100 degree-days, production of target Nile tilapia fry per gram of female also increased with increased degree-days ($Y = 0.007x - 0.43$, $r^2 = 0.428$, $p \leq 0.01$). The percentage of total Nile tilapia fry production retained by the grader, i.e., too large for sex reversal, increased significantly with accumulated degree days above 190 degree days ($Y = 0.52x - 100.57$, $r^2 = 0.564$, $p \leq 0.01$).

Blue tilapia fry production did not occur at less than 115 degree days. Total blue tilapia fry production per gram of female broodfish increased significantly with degree days above 100 degree days ($Y = 0.016x - 1.832$, $r^2 = 0.469$, $p \leq 0.01$). Production of target blue tilapia at greater than 100 degree days increased significantly with accumulated degree days ($Y = 0.01x - 0.871$, $r^2 = 0.325$, $p \leq 0.01$). Above 190 degree days, the percentage of the blue tilapia fry population retained by the grader increased significantly with increased cumulative degree days ($Y = 0.463x - 89.698$, $r^2 = 0.481$, $p \leq 0.01$).

PROGENY TESTING TO IDENTIFY YY MALE TILAPIA

Ronald P. Phelps
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, U.S.A.

Abstract

Sex determination in *Oreochromis niloticus* has been described as an XX female:XY male system in which the presence of a Y chromosome establishes the male sex. If a genotypic male is sex reversed to a phenotypic female, and if that phenotypic (XY) female is crossed with a normal male, a portion of the offspring will be of the YY genotype. Fish of this genotype (YY supermales) would produce only Y sperm and produce only male offspring if this inheritance pattern holds true. However, a supermale can only be identified by examining the sex ratio of his offspring.

Past research initiated a breeding program which resulted in the production of several families with different sex ratios. Three of these families had 3:1 male to female sex ratios which could be expected from a cross of a XY female to a XY male producing viable YY offspring. Another three families exhibited a 2:1

male:female sex ratio, a result which suggests a lethal YY genotype. Males from these families were mated with normal females and the sex ratio of their offspring determined. If the theoretical model holds true, the observed sex ratio should be close to the theoretical sex ratio. A second set of experiments consisted of spawning males from families that produced more than 99% male offspring. In addition, two individual males, identified in the 1993 spawning season as producing more than 95% male offspring and therefore being potential supermales, were spawned again in the 1994 season to check the constancy of reproductive results.

Males from the three families with a 3:1 male:female sex ratio produced progeny with a mean percentage of 57.4% males. Only 11% of the tested males produced more than 95% male progeny. The expected values for a XX:XY inheritance pattern would have been 66.7% for the mean percentage and 33.3% for males that produce more than 95% male offspring. Males from the 2:1 male:female families produced 54.2% male progeny and 8.3% of the tested males produced more than 95% male offspring. Males from the families that produced more than 99% male offspring produced 43.4% male progeny. None of these males produced more than 95% male progeny.

These distributions are similar to that of the parent population which was used as a control and for which the values were 54.1% and 5.3%, respectively. In this parent population, individual males were identified that sired more than 95% male progeny. Repeat spawning of these individuals resulted in varying sex ratios, contrary to what would be expected from a XX:XY inheritance. Male "A" produced five sets of progeny; three were 100% male, one was 83% male, and one was 60% male. Male "B" produced four sets of progeny with the following sex composition: 63, 63, 74, and 95% males.

The results of these studies indicate that sex inheritance in the Ivory Coast strain of *O. niloticus* cannot be explained by a XX:XY system. The variance from the expected ratios suggests control mechanisms from both the male and the female genome. Such contributions complicate a YY supermale breeding program and will require additional selection to obtain true breeding lines.

BINDING SITES FOR THE MASCULINIZING STEROID MIBOLERONE IN THE GONADAL TISSUE OF ADULT NILE TILAPIA (OREOCHROMIS NILOTICUS)

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Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, U.S.A.

Abstract

A commonly used masculinizing agent in international aquaculture is 17α-methyltestosterone (MT). Although procedures are well established for using steroids as sex inverting agents, the physiological mechanism of action of these steroids is still unknown. Past studies have demonstrated that MT accumulates in the liver, digestive tract, kidney, and gonadal tissues of tilapia. The presence of MT in gonadal tissue and the profound changes that must occur in order to direct differentiation towards the development of testes suggests that they may be target organs for masculinizing steroids. The classical model of steroid action is through an intracellular receptor that once bound to a steroid acts as a transcription factor.

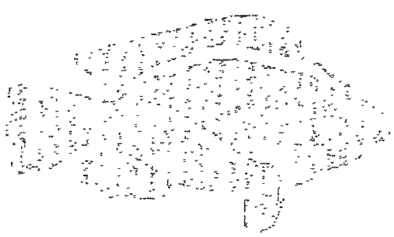
A binding site in the gonadal tissue of adult Nile tilapia (*Oreochromis niloticus*) was characterized using the synthetic androgen mibolerone (17-hydroxy-7,17-dimethylestr-4-en-3-one). The binding site demonstrated high affinity ($K_d = 1.03 \pm 0.11$ nM ; n=2) and low capacity ($B_{max} = 5.65 \pm 0.42$ fmol/mg protein; n=2) for mibolerone binding. Furthermore, it was located in gonadal cytosol only. The binding site also demonstrated ligand specificity. Only steroids with sex inverting capabilities displaced tritiated mibolerone binding. In addition a receptor assay based on the binding characteristics of different sex inverting agents was developed to determine in a fast and effective way the sex inverting potential of new synthetic steroids.

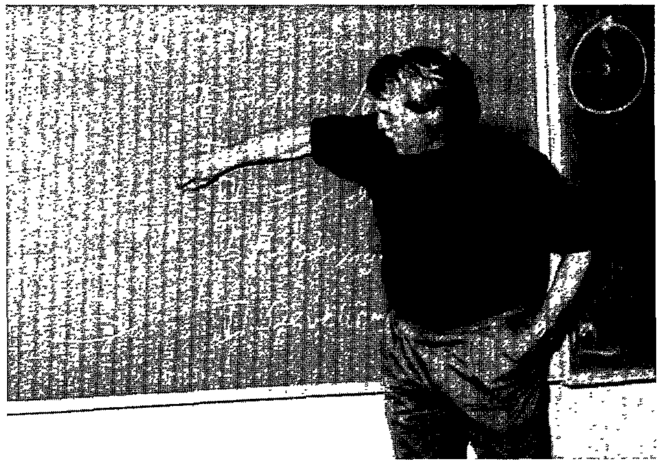
IMMERSION OF NILE TILAPIA IN 17α-METHYLTESTOSTERONE AND 17α-METHYLDIHYDROTESTOSTERONE FOR THE PRODUCTION OF ALL-MALE POPULATIONS

William L. Gale, Martin S. Fitzpatrick,
and Carl B. Schreck
Oregon Cooperative Fisheries Research Unit
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, U.S.A.

Abstract

Although hormone therapy for sex control has become nearly routine in aquaculture, there is room for improvement in selecting the safest and most effective hormones, as well as in developing procedures to minimize human and environmental exposure. The effectiveness of steroid immersion for masculinization of tilapia (*Oreochromis niloticus*) fry was examined. A 12-day exposure (between 10 and 22 days post-fertilization) to 17α-methyltestosterone (MT; 1000, 100, or 10 μg/l) was used initially, but resulted in high mortality and was ineffective in masculinizing the survivors. Factors such as paradoxical feminization and stress associated with steroid exposure and crowding may explain these observations. Further studies were conducted using 3-hr immersions in MT or 17α-methyldihydrotestosterone (MDHT) at 500 or 100 μg/l administered at 10 and 13 days post-fertilization. Mortality was considerably reduced with this treatment scheme as compared to the 12-day exposure. In one trial in which controls were 48% males, MDHT at 500μg/l produced 100% males; MDHT at 100 μg/l produced 78% males; MT at 500 μg/l produced 73% males; and MT at 100 μg/l produced 80% males. These results suggest that immersion offers potential as an alternative method for masculinization of tilapia and that further studies are warranted.





William Shelton (University of Oklahoma) lectures on black carp reproduction during a seminar at CLAR.

EFFECT OF 17α-METHYLTESTOSTERONE ON THE GROWTH OF TWO TILAPIA SPECIES, OREOCHROMIS AUREUS AND OREOCHROMIS MOSSAMBICUS, IN FRESH WATER

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Hawaii Institute of Marine Biology
University of Hawaii at Manoa
Kaneohe, Hawaii, U.S.A.

Abstract

We examined the effect of 17α-methyltestosterone (MT) on the growth of two tilapia species, *Oreochromis aureus* and *Oreochromis mossambicus*, reared in freshwater. The growth rate of *O. aureus* was nearly twice that of *O. mossambicus* at each dose level (0, 1, 10, and 25 mg of MT/kg of feed). With the exception of *O. aureus* treated with 1 mg of MT/kg of feed, MT treatment significantly increased ($p \leq 0.01$) growth in both species over control animals. In *O. mossambicus*, growth performance increased with increased levels of MT. By contrast, the 10 and 25 mg of MT/kg of feed treatments stimulated growth equally in *O. aureus*. The gonadosomatic index (GSI) was not significantly different between treatments within each species. It was, however, significantly lower ($p \leq 0.0001$) in *O. aureus* than in *O. mossambicus*. Gonadal weights were not significantly different between species, which

suggests that the smaller GSI in *O. aureus* results, at least in part, from the larger somatic mass of the animals. In both species, the hepatosomatic index (HSI) and absolute liver weight tended to increase with increased levels of MT and were significantly greater ($p \leq 0.05$; $p \leq 0.01$) in the 25 mg of MT/kg of feed treatment groups than in controls. The male-to-female sex ratio was not significantly different from 1:1 in any treatment group in either species.

ADDITIONAL ACHIEVEMENTS: PUBLIC SERVICE AND COMMUNITY DEVELOPMENT

As a research program, the focus of the CRSP in Egypt was the generation of technical information; however, the achievements of the Egypt project encompass more than the successful completion of experiments. This is largely due to ancillary activities associated with the Egypt project. The collaboration between Egypt and the CRSP resulted in the strengthening of institutional capacities, the development of new linkages, and increased educational opportunities for Egyptian scientists and farmers in the Nile delta region.

Main entry into the Central Laboratory for Aquaculture Research at Abbassa, Egypt.

BOLSTERING INSTITUTIONS

In Egypt, the CRSP assisted with facility maintenance. Ponds at the Central Laboratory for Aquaculture Research in Abbassa were renovated to prepare them for the start of the CRSP experiments. The CRSP also supported the refurbishing of an over-wintering facility for tilapia. Egyptian aquaculture and fish research benefited from the collaboration of U.S. and Egyptian scientists, who developed a comprehensive list of 26 journals on aquaculture, fisheries, and aquatic environments to be included as serial acquisitions for the new library of the National Agricultural Research Project. The CRSP further contributed to the library at the Central Laboratory for Aquaculture Research by sponsoring two-year subscriptions to several journals. The visits of CRSP researchers in Egypt also enlarged the institutional and professional network available to students and faculty, strengthening both U.S. and Egyptian universities through these increased international linkages.

AMPLIFYING RESULTS

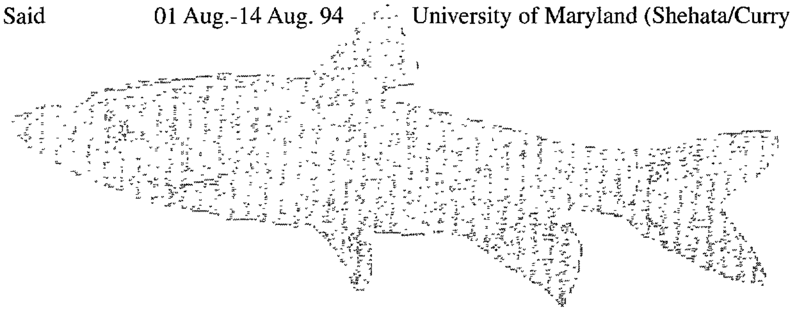
Formal training programs have not been funded by the CRSP Egypt project; nevertheless, the involvement of students constitutes an important part of the CRSP's international outreach. Ten students were supported by the Egypt project; six were working on advanced degrees, while four others conducted research at the University of Hawaii, at Auburn University, and at Oregon State University.

Scholarly Exchange Program

The centerpiece of the CRSP's professional development activities in Egypt was the scholarly exchange program. Twenty Egyptian researchers participated in this program. Egyptian scientists visited CRSP counterparts at their home institutions in the U.S. and abroad. Collaborative research was conducted at Oregon State University, Auburn University, the University of Oklahoma, the University of Hawaii, and the Asian Institute of Technology in Thailand. In addition, the Scholarly Exchange Program supported the attendance of CRSP Egyptian scientists at prominent scientific meetings,

Scholarly Exchange Program Overview

Name	*Dates*	*Destination (host)*	*Purpose*
Abdel R. El Gamal	16 Mar.-31 Mar. 93	University Oklahoma (Shelton),	collaborative visit
		CRSP Annual Meeting	CRSP Ann. Meeting
		Oregon State University (Goetze, Fitzpatrick)	collaborative visit
		Auburn University (Duncan)	collaborative visit
Ahmed Said Deyab	16 Mar.-31 Mar. 93	University Oklahoma (Shelton),	collaborative visit
		CRSP Annual Meeting	CRSP Ann. Meeting
		Oregon State University (Goetze, Fitzpatrick)	collaborative visit
		Auburn University (Duncan)	collaborative visit
Fatma Hafez	23 May-31 May 93	Torremolinos, Spain	WAS meeting
Gamal El Nagar	23 May-31 May 93	Torremolinos, Spain	WAS meeting
Ali Abdelghany	02 Oct.-09 Oct. 93	Hobart, Tasmania	Int'l Symp. on Fish Nutr.
Abdel Rahman Mostafa	05 Dec.-18 Dec. 93	Asian Institute of Technology, Thailand (Lin)	collaborative visit
Fatma Hafez	11 Jan.-19 Jan. 94	New Orleans	WAS meeting
Yassir Amad	11 Jan.-19 Jan. 94	New Orleans	WAS meeting
Ibrahim Shaker	11 Jan.-19 Jan. 94	New Orleans	WAS meeting
Hussein El Ghobashy	11 Jan.-19 Jan. 94	New Orleans	WAS meeting
Ali Abdelghany	11 Jan.-19 Jan. 94	New Orleans	WAS meeting
	19 Jan.-28 Jan. 94	NW Fisheries Science Center, Seattle (Hardy)	collaborative visit
Hussein Hebicha	30 Jan.-11 Feb. 94	Auburn University (Hatch)	collaborative visit
Ashraf Soliman	06 Mar.-19 Mar. 94	University of Oklahoma (Shelton)	collaborative visit
Hani Ebrahim	06 Mar.-18 Mar. 94	University of Hawaii (Grau)	collaborative visit
Ali Abdelghany	26 Mar-04 Apr. 94	Hilo, Hawaii	CRSP Ann. Meeting
	04 Apr.-08 Apr. 94	University of Hawaii (Grau)	collaborative visit
Zeinab Elnagdy	26 Mar-01 Apr. 94	Hilo, Hawaii	CRSP Ann. Meeting
	01 Apr.-16 Apr. 94	Auburn University (Boyd)	collaborative visit
Hussein El Ghobashy	08 May-21 May 94	Auburn Universiy (Dunham)	collaborative visit
Gamal A. Naser Mohamed	24 Jul.-05 Aug. 94	Oregon State University (Fitzpatrick)	collaborative visit
Khaled H. Hassan	24 Jul.-05 Aug. 94	Oregon State University (Fitzpatrick)	collaborative visit
Samir Ali Zein Elabedeen	01 Aug.-13 Aug. 94	Auburn University (Phelps)	collaborative visit
Ahmed Mostafa Khater	01 Aug.-13 Aug. 94	Auburn University (Phelps)	collaborative visit
Waheed Elwan Gadallan	01 Aug.-13 Aug. 94	Auburn University (Phelps)	collaborative visit
Nema A. Fatah Ali	01 Aug.-14 Aug. 94	University of Maryland (Shehata/Curry Woods)	collaborative visit
Samir Mohamed Said	01 Aug.-14 Aug. 94	University of Maryland (Shehata/Curry Woods)	collaborative visit



including the World Aquaculture Society conferences in both Spain and New Orleans, which promoted and facilitated Egypt project research.

Workshops and Seminars at the Central Laboratory for Aquaculture Research

As the collaborative process matured, workshops and seminars evolved to become an important component of the CRSP Egypt project. They provided a unique opportunity for information exchange that was specifically responsive to the needs of the attendees. Over 160 individuals (CRSP participants, outside researchers, farmers, and other interested people) participated in CRSP-sponsored workshops at the Central Laboratory for Aquaculture Research at Abbassa.

Workshops and seminars were conducted at Abbassa on a wide variety of topics, for example:

— Reproductive biology of fishes, a series of seminars and laboratory presentations covering reproductive physiology of fishes, induced breeding in fishes, sex differentiation in tilapia, sex identification of tilapia fry, and physiological sampling methods.

— Tilapia fingerling production, including field demonstrations of spawning pond harvest, broodfish handling, fry collection, handling and transport, and sex reversal treatment.

— Water quality management in aquaculture ponds, a two-day workshop which emphasized the influence of pond soils on water quality parameters.

— An Egypt CRSP field day was held at CLAR to highlight project progress and results to USAID and Government of Egypt personnel. Participants were informed about the status of the experiments and given a tour of the facility.

— An international workshop providing in-service training for Egyptian government aquaculture/ fisheries personnel covered a variety of topics. In an unprecedented move made possible through the contacts of a CRSP scientist, a leading scientist from Israel was invited by the Government of Egypt to participate in the workshop.

Linkage Development

The collaboration between the Central Laboratory for Aquaculture Research and the CRSP enabled both institutions to widen and strengthen their professional networks. The most visible success in this area is ICLARM's current interest in moving its research activities to the Central Laboratory for Aquaculture Research. Visiting CRSP scientists attended workshops at CLAR presented by University of Washington scientists discussing fish health management and nutrition, as well as a University of Arizona scientist's workshop on cage culture and integration of fish culture with irrigation water. A visit to CLAR by a U.S. Fish and Wildlife Service employee resulted in establishing communications with a group of fish farmers that had never previously communicated with CLAR or the CRSP. CRSP researchers also visited the Mariut Fish Farm and met with members of the Oceanic Institute. CRSP scientists discussed black carp studies with faculty of Zagazig University. The Director of the Aquatic Animal Health Research Institute in Thailand presented a seminar about diseases of cultured fish. Other connections included the Northwest Fisheries Science Center in Seattle and the University of Maryland.

These professional interactions contributed to a global information exchange, helped to increase the visibility of both the CRSP and CLAR, and promoted cooperation in research efforts.

ENDURING CONTRIBUTIONS:

CRSP Central Database

The standardized data collected during the Egypt project's Global Experiment research will be added to the CRSP Central Data Base. The CRSP Central Data Base contains standardized records from all CRSP research sites; thus, it provides opportunities for many kinds of global analyses. The data base was designed to facilitate communication with other large data bases, thereby creating opportunities for collaboration. CRSP scientists, as well as scientists in the aquaculture community at large, may contribute to and access the data base. To further broaden the use of and access to the database, the CRSP is currently collaborating with ICLARM to integrate the CRSP database with ICLARM's *FISHBASE*.

The Egypt data are particularly valuable because they represent a climate thus far not represented in the CRSP database. This will provide a new basis for comparisons between ponds. Further, information in the data base will be of use to the CRSP Data Analysis and Synthesis Team and will be incorporated into pond models. In this way the Egypt data will be of ongoing value to the CRSP and the aquaculture community. The result will be a deeper and more complete understanding of pond dynamics.

Economic Returns

The CRSP Egypt project generated substantial benefits to the U.S. as well as to Egypt. Not only did participating U.S. researchers benefit from collaborating with their Egyptian counterparts, but U.S. products and services were extensively involved in the project. Due to bilateral agreements, Egyptian scientists were able to purchase scientific equipment without having to pay duty and/or other fees. Over $123,635 was spent on equipment and airfare provided by U.S. companies. Although not a direct goal of the project, these "ripple" effects contribute to the wide-reaching positive influence of the CRSP project.

INSTITUTIONAL COLLABORATION

Research projects were conducted by members of the following institutions:

Auburn University, Auburn, Alabama, U.S.A.
Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad, Sharkia, Egypt
Oregon State University, Corvallis, Oregon, U.S.A.
University of Hawaii, Hilo, Hawaii, U.S.A.
University of Michigan, Ann Arbor, Michigan, U.S.A./Asian Institute of Technology, Bangkok, Thailand
University of Oklahoma, Norman, Oklahoma, U.S.A.

Other institutional connections (through participation in the CRSP Technical Committee) involved the following U.S. universities:

Michigan State University, East Lansing, Michigan, U.S.A.
University of Arkansas at Pine Bluff, Pine Bluff, Arkansas, U.S.A.
University of California, Davis, California, U.S.A.

The Central Laboratory for Aquaculture Research falls under the authority of the Agricultural Research Center, Ministry of Agriculture and Land Reclamation.

Workshop participants at the Central Laboratory for Aquaculture Research in Abbassa, listening to presentations from visiting CRSP scientists.

207

PROJECT PARTICIPANTS

The Egypt project of the Pond Dynamics/Aquaculture CRSP represents the joint effort of 61 researchers, technicians, students, and support personnel from Egypt, the U.S.A., and Thailand. Twelve Egyptian scientists collaborated with twelve U.S. and one Thai researcher. The expertise of host country and U.S. personnel is broad-based and includes, but is not limited to, the following fields of specialization: general aquaculture, fisheries, limnology, water quality, biotechnology, physiology, nutrition, fish health, data management, soil science, chemistry, biology, social science, economics, and administration.

		FIELDS OF SPECIALIZATION					
Individual	CRSP Function	Research Administration	Limnology/ Water Quality	Fisheries/ Aquaculture	Data Management	Social Sciences	Location of Work (1)
		MANAGEMENT ENTITY					
Hillary Egna	Director	X	X	X			Corvallis, Oregon
Brigitte Goetze	Deputy Director and Egypt Coordinator	X	X				Corvallis, Oregon
Marion McNamara	Assistant Director	X					Corvallis, Oregon
Naomi Weidner	Admin. Assistant	X					Corvallis, Oregon
		AUBURN UNIVERSITY					
Claude Boyd	U.S. Co-Principal Investigator		X	X			Auburn, Alabama
Bryan Duncan	U.S. Co-Principal Investigator	X		X			Auburn, Alabama
Bartholomew Green	U.S. Co-Principal Investigator	X	X	X	X		Abbassa, Egypt
Ronald Phelps	Researcher		X	X			Auburn, Alabama
Valentin Abe	Graduate Research Assistant		X	X			Auburn, Alabama
Shivaun Leonard	Graduate Research Assistant			X			Auburn, Alabama
Prasert Munsuri	Graduate Research Assistant		X	X			Auburn, Alabama
P. Parks	Fiscal Officer	X					Auburn, Alabama

(1) Denotes primary work location and excludes host country site visits and travel for attendance of meetings.

Individual	CRSP Function	FIELDS OF SPECIALIZATION: Research Administration	Limnology/ Water Quality	Fisheries/ Aquaculture	Data Management	Social Sciences	Location of Work (1)
CENTRAL LABORATORY FOR AQUACULTURE RESEARCH							
Abdel Rahman El Gamal	H.C. Principal Investigator	X	X	X			Abbassa, Egypt
Ali E. Abdelghany	Researcher	X		X			Abbassa, Egypt
Hussein El Ghobashy	Researcher	X		X			Abbassa, Egypt
Gamal El Nagar	Researcher			X			Abbassa, Egypt
Fatma El Nemaky	Researcher				X		Abbassa, Egypt
Abdel Moez Faried	Researcher		X				Abbassa, Egypt
Fatma Hafez	Researcher			X			Abbassa, Egypt
Hussein Hebicha	Researcher				X		Abbassa, Egypt
Esam Hosny Rizkalla	Researcher			X			Abbassa, Egypt
Abdel Rahman Mostafa	Researcher			X			Abbassa, Egypt
Zeinab Attia Nagdy	Researcher		X				Abbassa, Egypt
Ahmed Said	Researcher			X			Abbassa, Egypt
Ahmed Khater	Research Associate			X			Abbassa, Egypt
Gamal Abdel Nasser	Research Associate			X			Abbassa, Egypt
Ibrahim Shaker	Research Associate		X				Abbassa, Egypt
Ahmed Abdel Fatah	Research Assistant			X			Abbassa, Egypt
Safwat Abdel Ghany	Research Assistant		X				Abbassa, Egypt
Khalid Hussein	Research Assistant			X			Abbassa, Egypt
Nabil Ibrahim	Research Assistant		X				Abbassa, Egypt
Dia A. R. Kenawy	Research Assistant		X				Abbassa, Egypt
Mohamed Abdel Salam	Research Assistant			X			Abbassa, Egypt
Naem Shamees	Research Assistant			X			Abbassa, Egypt
Namat Abdel Fatah	Chemist		X				Abbassa, Egypt

(1) Denotes primary work location and excludes host country site visits and travel for attendance of meetings.

Individual	CRSP Function	Research Administration	Limnology/ Water Quality	Fisheries/ Aquaculture	Data Management	Social Sciences	Location of Work (1)
		FIELDS OF SPECIALIZATION					
CENTRAL LABORATORY FOR AQUACULTURE RESEARCH (continued)							
Samir Said	Chemist		X				Abbassa, Egypt
Mona Hamed	Biologist		X				Abbassa, Egypt
Mostafa Abdel Mohsen	Biologist		X				Abbassa, Egypt
Samir Zain Elabeden	Technical Engineer			X			Abbassa, Egypt
Wahied Elwan	Technical Engineer			X			Abbassa, Egypt
Tharwat Ismail	Technical Engineer			X			Abbassa, Egypt
Ahmed Nassralla	Technical Engineer			X			Abbassa, Egypt
Ashraf Soluman	Technical Engineer			X			Abbassa, Egypt
Mohamed Wafik	Technical Engineer		X				Abbassa, Egypt
Seham Ahmed	Laboratory Assistant		X				Abbassa, Egypt
Mahmoud Abou El Nour	Accountant	X					Abbassa, Egypt
OREGON STATE UNIVERSITY							
Martin Fitzpatrick	U.S. Co-Principal Investigator	X		X			Corvallis, Oregon
Carl Schreck	U.S. Co-Principal Investigator	X		X			Corvallis, Oregon
William Gale	Graduate Research Assistant			X			Corvallis, Oregon
Robert Halvorsen	Fiscal Officer	X					Corvallis, Oregon
UNIVERSITY OF HAWAII							
Gordon Grau	U.S. Principal Investigator	X		X	X		Kaneohe, Hawaii
Kevin Hopkins	U.S. Principal Investigator	X		X	X		Hilo, Hawaii
Hal Richmond	Research Assistant			X	X		Kaneohe, Hawaii
Steve Shimoda	Research Assistant	X		X			Kaneohe, Hawaii
Bo Alexander	ResearchAssistant			X			Kaneohe, Hawaii
A. Chang	Fiscal Officer	X					Kaneohe, Hawaii

(1) Denotes primary work location and excludes host country site visits and travel for attendance of meetings.

Individual	CRSP Function	Research Administration	Limnology/ Water Quality	Fisheries/ Aquaculture	Data Management	Social Sciences	Location of Work (1)
		FIELDS OF SPECIALIZATION					
		UNIVERSITY OF MICHIGAN					
James Diana	U.S. Principal Investigator	X		X			Ann Arbor, Michigan
P. Stemple	Fiscal Officer	X					Ann Arbor, Michigan
		UNIVERSITY OF MICHIGAN/ASIAN INSTITUTE OF TECHNOLOGY					
C. Kwei Lin	Principal Investigator		X	X			Bangkok, Thailand
		UNIVERSITY OF OKLAHOMA					
William Shelton	U.S. Principal Investigator			X			Norman, Oklahoma
B. Quinn	Fiscal Officer	X					Norman, Oklahoma

(1) Denotes primary work location and excludes host country site visits and travel for attendance of meetings.

Staff at the Central Laboratory for Aquaculture Research at Abbassa take a seine sample for a mid-term evaluation of fish growth and survival in the polyculture experimental ponds.

207

FINANCIAL SUMMARY

The expenditure of funds for research and project management activities is summarized in the following table. Funding was provided under a grant from USAID/Cairo and the National Agricultural Research Project of Egypt (USAID Grant No. 263-0152-G-00-2231-00) and by the participating institutions, although cost-sharing was not required.

Research activities were conducted during the period from 1 October 1992 through 31 December 1994. Data analysis and report writing occurred during the period from 1 January 1995 through 31 March 1995. The project-end-date was 31 March 1995. This unaudited summary is still preliminary; a final financial report will be submitted to USAID by the Management Entity 90 days after the project end date.

EGYPT PROJECT ESTIMATED EXPENDITURES FROM 1 OCT. 92 THROUGH 31 MAR. 95

	USAID allocations[1,2]	USAID funds expended[1]	Voluntary contribution [3]	Total expenses
U.S.A.-based activities				
Research conducted in Egypt:				
Auburn University	$513,274	$508,798	$25,009	$533,807
University of Oklahoma	$111,596	$107,980	$20,883	$128,863
Subtotal	$624,870	$616,778	$45,892	$662,670
Research conducted in Thailand:				
University of Michigan/AIT	$24,684	$24,176	$7,836	$32,012
Subtotal	$24,684	$24,176	$7,836	$32,012
Research conducted in U.S.A.:				
Auburn University	$11,004	$11,004	$550	$11,554
University of Hawaii	$65,323	$65,300	$13,515	$78,815
Oregon State University	$110,906	$112,177	$25,497	$137,674
Subtotal	$187,233	$188,481	$39,562	$228,043
Management Entity				
Program Management [4]	$379,119	$375,093	$24,570	$399,663
Total U.S.A.	*$1,215,906*	*$1,204,528*	*$117,860*	*$1,322,388*
Transferred funds [5]	$125,600	$120,954	n.a.	$120,954
Total U.S.A. plus transferred funds	*$1,341,506*	*$1,325,482*	*$117,860*	*$1,443,342*
Egypt-based activities				
Total Egypt (incl. transfers) [6]	*$369,569*	*$369,569*	*n.a.*	*$369,569*
Total project [7]	**$1,585,475**	**$1,574,096**	**$117,860**	**$1,691,956**

Notes:

1 Values include subcontract charges

2 Based on obligations under grant no. 263-0152-G-00-2231-00

3 Estimates of U.S. university voluntary contributions

4 Also includes technical publications for the entire project and library support for CLAR

5 Transfers from the Egyptian portion of the budget to the U.S. portion of the budget were initiated to expedite travel authorizations and equipment purchases

6 Estimate based on original grant document budget

7 The sum of "Total U.S.A." and "Total Egypt"

208